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Transporter expression in non-cancerous and cancerous liver tissue from donors with hepatocellular carcinoma and chronic hepatitis C infection quantified by LC-MS/MS proteomics.

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a) Running Title: Transporter expression in HCV-hepatocellular carcinoma tissues

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d) Abbreviations: bile salt export pump (BSEP), breast cancer resistance protein (BCRP), cancerous (C), chronic hepatitis C infection (HCV), gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA), hepatitis C infected subject with hepatocellular carcinoma (HCV-HCC), hepatocellular carcinoma (HCC), international normalized ratio (INR), liquid chromatography tandem mass spectrometry (LC-

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MS/MS), liver tissue cell distribution system (LTCDS), magnetic resonance imaging (MRI), multidrug and toxin extrusion protein (MATE), multidrug resistance-associated protein (MRP), non-cancerous (NC), organic anion transporting polypeptide (OATP), organic cation transporter (OCT), P-glycoprotein (P-gp), physiologically based pharmacokinetic modelling (PBPK), single nucleotide polymorphisms (SNP) and sodium/taurocholate co-transporting polypeptide (NTCP).

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Abstract

Protein expression of major hepatobiliary drug transporters (NTCP, OATPs, OCT1, BSEP, BCRP, MATE1, MRPs and P-gp) in cancerous (C, n=8) and adjacent non-cancerous (NC, n=33) liver tissues obtained from chronic hepatitis C patients with hepatocellular carcinoma (HCV-HCC) were quantified by LC-MS/MS proteomics. Herein, we compare our results with our previous data from non-infected non-cirrhotic (control, n=36) and HCV-cirrhotic (n=30) livers. The amount of membrane protein yielded from NC and C HCV-HCC tissues decreased (31%, 67%) relative to control livers. In comparison to control livers, with the exception of NTCP, MRP2 and MATE1, transporter expression decreased in NC (38-76%) and C (56-96%) HCV-HCC tissues. In NC HCV-HCC tissues NTCP expression increased (113%), MATE1 expression decreased (58%) and MRP2 expression was unchanged relative to control livers. In C HCV-HCC tissues, NTCP and MRP2 expression decreased (63%, 56%) and MATE1 expression was unchanged relative to control livers. Compared to HCV-cirrhotic livers, aside from NTCP, OCT1, BSEP and MRP2, transporter expression decreased in NC (41-71%) and C (54-89%) HCV-HCC tissues. In NC HCV-HCC tissues, NTCP and MRP2 expression increased (362%, 142%), whilst OCT1 and BSEP expression were unchanged. In C HCV-HCC tissues, OCT1 and BSEP expression decreased (90%, 80%) relative to HCV-cirrhotic livers, while NTCP and MRP2 expression were unchanged. Expression of OATP2B1, BSEP, MRP2 and MRP3 decreased (56-72%) in C HCV-HCC tissues in comparison to matched NC tissues (n=8) but the expression of other transporters was unchanged. These data will helpful in future to predict transporter-mediated hepatocellular drug concentrations in patients with HCV-HCC.

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Introduction

The world health organisation estimated globally 71 million people had HCV and over 100000 people with HCV died as a result of HCC in 2015 (World Health Organisation, 2017). Over 90% of HCV patients treated with direct acting antiviral drug combinations achieve clearance of circulating HCV RNA or a sustained virological response, a result considered to be a functional cure (Bonaventura and Montecucco, 2016; Terrault et al., 2016). Despite this success, the incidence of diagnosis and treatment of HCV is low because up to 90% of people infected are unaware of their infection status (Institute of Medicine Committee on the Prevention and Control of Viral Hepatitis, 2010; Hatzakis et al., 2011). The incidence of liver cirrhosis 25 to 30 years after HCV-infection ranges from 15 to 35% (Freeman et al., 2001). Once HCV-related cirrhosis is established, hepatocellular carcinoma (HCC) typically develops at an annual rate of 1 to 4% (Hassan et al., 2002; Blonski and Reddy, 2008); although rates of up to 8% have been reported in Japan (El-Serag, 2012).

Cross-sectional and case control studies have found a strong association between chronic HCV-infection and HCC (Goodgame et al., 2003). However, the cause of carcinogenesis is unclear. An important clinical observation is that HCC in patients with HCV predominantly occurs in patients with advanced stages of hepatic fibrosis or cirrhosis (Blonski and Reddy, 2008; Lok et al., 2009; El-Serag, 2012; Morgan et al., 2013). Diagnosis of HCC is based upon imaging techniques and/or biopsy. The hepatocyte-specific magnetic resonance imaging (MRI) contrast agent Gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA) was developed to detect and characterise focal liver lesions (Schuhmann-Giampieri et al., 1992; Hamm et al., 1995). The hepatobiliary transport of Gd-EOB-DTPA is mediated by organic-anion transporting polypeptides 1B1 and 1B3 (OATP1B1, OATP1B3), Na-taurocholate co-transporting polypeptide (NTCP) and multidrug resistance-associated protein 3 (MRP3) located at the sinusoidal membrane and multidrug resistance-associated protein 2 (MRP2) situated at the canalicular membrane of hepatocytes (Leonhardt et al., 2010; Jia et al., 2014). During MRI,

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hepatic focal lesions that have reduced uptake transporter expression are depicted as hypo-intense areas compared with the well-enhanced liver tissue with normal hepatobiliary function (Narita et al., 2009; Kitao et al., 2010; Tsuboyama et al., 2010; Vasuri et al., 2011).

Up to 90% of HCC patients are unable to undergo curative liver resection or receive a liver transplant (Colella et al., 1998; Fan et al., 1999; Fong et al., 1999). Drug resistance often hampers successful treatment of HCC with chemotherapeutic agents. Such resistance can be conferred by various mechanisms such as: (i) reduced uptake of the drug into the target cells; (ii) alterations within the cells such as altered metabolism, an increased capacity to repair DNA or reduced apoptosis; and (iii) an increased efflux of the drug from the target cells (Holohan et al., 2013). Drugs such as doxorubicin, cisplatin or epirubicin used in transarterial chemoembolization therapy (i.e., arterial administration of chemotherapy followed by embolization of the arterial blood supply) and the oral kinase inhibitor sorafenib are known substrates of uptake and efflux transporters. Doxorubicin is a substrate of the efflux transporters P-glycoprotein (P-gp), breast cancer resistance protein (BRCP) and MRP1 (Fairchild et al., 1987; Wei et al., 2012; Saeed et al., 2015). Cisplatin is a substrate of hepatic organic cation transporters (OCT1, OCT2 and OCT3) and multidrug and toxin extrusion protein 1 (MATE1) (Yonezawa et al., 2006). Epirubicin is a substrate of P-gp (Tariq et al., 2016). Sorafenib is a substrate of OATP1B1, OATP1B3, OCT1, P-gp and BCRP (Gnoth et al., 2010; Lagas et al., 2010; Swift et al., 2013; Zimmerman et al., 2013; Johnston et al., 2014).

Based on the above data, it is important to understand if transporter expression is down-regulated in HCV-HCC liver tissue in order to: (i) better design drugs to target HCC in HCV-infected patients; and (ii) predict transporter-mediated drug disposition (including hepatocellular concentrations) in patients with HCV-HCC. Current mRNA and immunohistochemical data on expression of plasma membrane transporters in cancerous (C) HCV-HCC and adjacent non-cancerous (NC) and liver tissues is controversial. Therefore,

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for the first time, we quantified the protein expression of major hepatobiliary transporters in C and NC HCV-HCC liver resections by targeted proteomics and LC-MS/MS using the surrogate peptide approach. Here, we compare the results obtained with our previously published data on transporter expression in non-infected non-cirrhotic (control, 21-70 yr.) and HCV-cirrhotic livers (33-69 yr.) (Prasad et al., 2016; Wang et al., 2016).

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Materials and Methods

Chemicals and Reagents

The ProteoExtract® Native Membrane Protein Extraction Kit was from Calbiochem (Temecula, CA). Iodoacetamide, dithiothreitol, trypsin and the BCA assay kit were from Pierce Biotechnology (Rockford, IL). Sodium deoxycholate was from MP Biochemicals (Santa Ana, CA). Bespoke synthetic signature peptides were from New England Peptides (Boston, MA). Equivalent stable isotope-labelled internal standards (AQUA QuantPro, ± 25% precision) and ammonium bicarbonate were from ThermoFisher Scientific (Rockford, IL). High-performance liquid chromatography-grade acetonitrile, methanol, and formic acid were from Fischer Scientific (FairLawn, NJ). Finally, deionized water was from a Q-Gard 2 purification cartridge water purifying system (Millipore, Bedford, MA). All reagents were analytical grade.

Procurement of human liver samples

Each liver sample was procured from patients undergoing liver transplant. The demographic and clinical characteristics of the liver donors are shown in Table 1. Gilead Sciences, Inc. provided twenty-five frozen NC HCV-HCC liver resection samples (classified by a pathologist). Eight paired NC and C HCV-HCC liver resection samples were obtained from the Liver Tissue Cell Distribution System (LTCDS), University of Minnesota. All the HCV-HCC and HCV-cirrhotic liver samples were flash frozen in liquid nitrogen within <1 hour of collection. The control (non-infected non-cirrhotic) liver samples were frozen within <24 hours of collection. All the liver samples were then stored at -80 °C until analysis. Demographics, acquisition and storage of the control and HCV-cirrhotic livers have been published previously (Prasad et al., 2016; Wang et al., 2016).

Membrane Extraction and Protein Trypsin Digestion

Total membrane was isolated from approximately 100 milligrams of HCV-HCC human liver tissue as previously described using the ProteoExtract® Native Membrane Protein

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Extraction Kit (Deo et al., 2012; Prasad et al., 2013; Prasad et al., 2014; Prasad et al., 2016; Wang et al., 2016). The isolated total membrane protein concentration of each sample was determined using the Pierce™ BCA protein assay kit. In triplicate, 2 µg/µL total membrane protein (80 µL) of each sample was reduced, denatured, alkylated, and digested according to our previously published protocol (Wang et al., 2015; Prasad et al., 2016; Wang et al., 2016). The peptide fragments generated by trypsin digestion were quantified by LC-MS/MS as described below.

Surrogate Peptide Selection and Quantification by LC-MS/MS

Unique surrogate peptides were selected for quantification of each transporter (Supplementary Table 1) on the basis of previously reported selection criteria (Kamiie et al., 2008; Prasad and Unadkat, 2014) and used as calibrators. Corresponding labelled peptides, at [$^{13}C_6{}^{15}N_2$]-lysine and [$^{13}C_6{}^{15}N_4$]-arginine residues, were used as internal standards. The calibrators, ranging from approximately 0.3 to 110 fmol (on-column), were prepared by spiking 50 mM ammonium bicarbonate buffer (80 µL) with unlabelled surrogate peptide standards (10 µL) and labelled internal standards (20 µL). The quality control samples were prepared by spiking surrogate peptides into 50 mM ammonium bicarbonate buffer (at low, medium and high concentrations). In addition, a biological quality control sample, membrane protein from a collection of control livers, was included in each assay to ensure that the data matched previously published data (Prasad et al., 2016).

Surrogate peptides were quantified using an AB Sciex™ 6500 TQS tandem mass spectrometer (AB SCIEX, Framingham, MA) coupled with a Water's Acquity UPLC system (Waters Corporation, Milford, MA) operated in electrospray positive ionization mode. The mass spectrometry conditions were; curtain gas: 20 psi, ion spray voltage: 5500 V, temperature: 350°C, Gas 1: 50 psi, Gas 2: 30 psi, and cell exit potential: 12 V.

Approximately, 8 µg of the trypsin digest (5 µL) was injected onto the column (Acquity UPLC® HSS T3 1.8µm 100A; 100 x 2.1 mm; Waters) fitted with a security guard column

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(C18, 4 x 2 mm; Phenomenex, Torrance, CA) and eluted at 0.3 mL/min with a gradient mobile phase consisting of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The linear gradient was; 0 – 3 minutes: 97% A 3% B, 3 – 10 minutes: 87% A 13% B, 10 –20 minutes: 75% A 25% B, 20 -22 minutes: 66.7% A 33.3% B, 22-23 minutes 50% A 50% B, 23-24 minutes 20% A 80% B, 24-28 minutes 97% A 3% B. MS/MS analysis was performed by monitoring the surrogate peptides and the internal standards using instrument parameters listed in Supplementary Table 1.

LC-MS/MS data were processed by integrating the peak areas generated from the reconstructed ion chromatograms for the analyte peptides and respective heavy internal standards using Analyst® Software 1.6.2 (Milford, MA). The peak response from two transitions of each peptide was averaged (after confirming that they were correlated) for quantification of samples, standards and quality controls. The calibration curve for each surrogate peptides were linear ($R^2 > 0.99$) with a lower limit of quantification ranging from 0.65 to 2.80 fmol (Supplementary Table 1). The accuracy (percentage error) and precision (coefficient of variation) of the assay, on the basis of the quality control samples, was 80-120% and <20%, respectively.

Genotyping Methods and Genotype-Dependent Changes in OATP1B1 protein expression.

We have previously shown correlation of some high frequency SLCO1B1 single nucleotide polymorphisms (SNPs) with OATP1B1 protein expression in adult liver samples. Therefore, all HCV-HCC liver tissues were genotyped for SLCO1B1 SNPs (rs4149015, -11187G>A; rs2306283, 388A>G; rs4149056, 521T>C; rs4149057, 571T>C), and rs2291075 (597C>T)) as previously described (Wang et al., 2016).

Statistical Data Analyses

Statistical difference ($p < 0.05$) in the expression of transporters between groups of livers was determined by the non-parametric Kruskal-Wallis and Dunn's multiple

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comparisons statistical test. Transporter expression in matched NC and C HCV-HCC tissues were assessed by the non-parametric Wilcoxon signed-rank test. Correlation in transporter expression within NC and C HCV-HCC tissues were determined by the non-parametric Spearman test.

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Results

Demographics

In the HCV-infected liver groups there was a male sampling bias (Table 1). This is likely because HCV prevalence is greater in men than women (Armstrong et al., 2006; Rantala and van de Laar, 2008). Many of the HCV-positive donors exhibited abnormal INR, alanine aminotransferase, aspartate aminotransferase, albumin and bilirubin serum levels indicating liver damage. Consistent with previous reports of chronic hepatitis, serum alkaline phosphatase concentrations were much less elevated than alanine aminotransferase and aspartate aminotransferase serum levels (Desmet et al., 1994). LTCDS were able to provide a full list of medications taken by each HCV-HCC subject but these data were not available for the Gilead samples (Supplementry Table 2).

Pooling of samples

Initially, total membrane protein yields and transporter expression levels (per µg of membrane protein and per g of liver) in NC HCV-HCC tissues from Gilead (n=25) and LTCDS (n=8) were assessed separately. No significant differences were observed between these two groups of livers ($p > 0.05$) for either total membrane protein yield or transporter expression level; therefore; the data from these two groups were pooled for all subsequent analyses detailed below.

Comparison of total membrane protein yield

Compared to control livers, the total membrane protein yield (per g of liver) from HCV-cirrhotic livers, NC or C HCV-HCC tissues was significantly lower by 33%, 31% and 67%, respectively (Figure 1A, $p < 0.05$). The total membrane protein yield from HCV-cirrhotic livers and NC HCV-HCC tissues was no different. The total protein yield from C HCV-HCC tissues was significantly lower than that from NC HCV-HCC tissues (52%, $p < 0.05$). In matched NC and C HCV-HCC tissues from the same person the total membrane protein

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yield from C tissues was also lower than NC tissues (46%, Figure 1B), but this didn't reach significance, likely due to the small sample size ($p = 0.055$).

Hepatobiliary transporter protein expression in control, HCV-cirrhotic, C and NC HCV-HCC human livers.

In order to predict human transporter-mediated drug disposition by physiologically based pharmacokinetic modelling (PBPK), protein expression of relevant transporters must be calculated per gram of liver tissue, then scaled up to the weight of an adult liver. The hepatic transporters quantified included sinusoidal uptake transporters NTCP, OATP1B1, OATP1B3, OATP2B1, and OCT1; sinusoidal efflux transporters MRP3 and MRP4; and canalicular efflux transporters BSEP, BCRP, MATE1, MRP2, MRP3 and P-gp. When transport protein expression was below the lower limit of quantification, the lower limit of quantification was used in calculations as an estimate of changes in transporter expression. The data are presented normalized to milligram of membrane protein and gram of liver (Figures 2 and 3).

Differences in the protein expression of major hepatobiliary transporters in HCV-cirrhotic livers versus control livers has been previously described (Wang et al., 2016) and is not recapitulated here. In comparison to control livers (on a per gram of liver basis), with the exceptions of NTCP, MRP2 and MATE1, transporter expression decreased in both NC (38-76%) and C (56-96%) HCV-HCC tissues. In NC HCV-HCC tissues, NTCP expression increased (113%), MATE1 expression decreased (58%), and MRP2 expression was no different to control livers. In C HCV-HCC tissues, NTCP and MRP2 expression decreased (63%, 56%) than control livers, and MATE1 expression was unchanged relative to control livers.

In comparison to HCV-cirrhotic livers (on a per gram of liver basis), with the exceptions of NTCP, OCT1, BSEP and MRP2, transporter expression also decreased in NC (41-71%) and C (54-89%) HCV-HCC tissues. In NC HCV-HCC tissues, expression of NTCP and MRP2 increased (362%, 142%) and expression of OCT1 and BSEP were unchanged

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relative to HCV-cirrhotic livers. In C HCV-HCC tissues, expression of OCT1 and BSEP decreased (90%, 80%), while NTCP and MRP2 expression were no different to HCV-cirrhotic livers. The only difference in transporter expression between NC and C HCV-HCC tissues (on a per gram of liver basis) was decreased expression of NTCP (83%) and MRP2 (73%) in C tissues. The outlined changes in transporter expression are summarized in Supplementry Table 3.

The impact of disease on transporter protein expression exhibited a transporter-dependent pattern, irrespective of whether it was normalized to milligrams of membrane protein or grams of liver. When transporter expression was normalized to milligram of membrane protein, expression of OATP1B1, OATP1B3, OCT1, BSEP and P-gp were decreased (33-66%), NTCP and MRP2 increased (192%, 126%) and OATP2B1, MATE1 and MRP3 unchanged in NC HCV-HCC tissues relative to control livers. In C HCV-HCC tissues, expression of OATPs OCT1 and BSEP were lower (46-88%) than control, while expression of NTCP, MATE1, MRP2, MRP3 and P-gp were no different. In comparison to HCV-cirrhotic livers, expression of OATPs, BSEP, MATE1, MRP3 and P-gp decreased (26-67%) NTCP and MRP2 increased (354%, 134%) and OCT1 expression was unchanged in NC HCV-HCC tissues. Whilst in C HCV-HCC tissues expression of OATP1B1, OATP2B1, OCT1, BSEP, MATE1 and MRP3 were decreased (49-81%) and NTCP, OATP1B3, MRP2 and P-gp were no different to HCV-cirrhotic livers. The outlined changes in transporter expression are summarized in Supplementry Table 4.

In all HCV-HCC tissues, expression of BCRP and MRP4 expression were below the lower limit of quantification (data not shown, LLOQ shown in Supplementary Table 1). In previous studies on control and HCV-cirrhotic livers, BCRP expression has been close to the LLOQ (Control: 14/36 <LLOQ, HCV-cirrhotic: 22/30 <LLOQ) and MRP4 expression has been below the LLOQ. A different instrument was used in this study in which the LLOQ for

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BCRP was higher. Therefore we cannot draw any reliable conclusions about the difference in BCRP expression.

Expression of each of the hepatobiliary transporter did not correlate with clinical markers of liver function listed in Table 1 ($r < 0.3$). However, multiple hepatic transporters exhibited significant protein–protein expression correlation in NC and C HCV-HCC tissues (Supplementary Figure 1, Spearman Coefficient (r) ≥ 0.4, $p < 0.05$).

Comparison of transporter expression in matched NC and C HCV-HCC liver tissues.

Expression of OATP2B1, BSEP, MRP2 and MRP3 (per gram of liver) in C HCV-HCC tissues was 56%, 66%, 72% and 66% lower than NC HCV-HCC tissues ($p < 0.05$, Figure 4). NTCP and OCT1 expression in C HCV-HCC tissues were also decreased (64% and 83%) relative to NC HCV-HCC tissues but this difference was found not to be statistically significant ($p = 0.06$). Expression of the remaining transporters was no different. Transporter expression in some of the matched C HCV-HCC tissues was below the lower limit of quantification (LLOQ shown in Supplementary Table 1). In these circumstances the lower limit of quantification was used as an estimate of changes in transporter expression.

Effect of SLCO1B1 phenotype on hepatic OATP1B1 transport protein expression

Previously we have observed genotype-dependent expression of OATP1B1, therefore all HCV-HCC tissues were genotyped for three key non-synonymous SLCO1B1 SNPs, c.388A>G, c.463C>A and c.521T>C. HCV-HCC tissues heterozygous for c.463C>A had higher OATP1B1 expression than the reference allele HCV-HCC tissues (Supplementary Figure 2A, $p < 0.01$). The remaining genotypes showed no significant difference in OATP1B1 expression, however the frequency of each allelic variant was low. Individual SNPs of SLCO1B1 show significant linkage disequilibrium (Kalliokoski et al., 2008; Nies et al., 2013; Prasad et al., 2014). Therefore, the samples were grouped based upon SLCO1B1

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haplotype (Supplementary Table 5). When normalized to gram of liver tissue, expression of OATP1B1 was significantly greater in heterozygotes with a *14 haplotype (Supplementary Figure 2B, $p < 0.05$). The above interpretations of OATP1B1 protein expression were not affected by genotype. When the tissue samples with *14 haplotype were removed the trends remained the same (data not shown).

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Discussion

This work, together with our previous reports on the protein expression of major hepatobiliary transporters in non-infected non-cirrhotic (control) and HCV-cirrhotic livers provide a comprehensive analysis of the impact of HCV-infection and HCV-HCC on hepatic drug transporter protein expression. HCV is a progressive fibrotic disease that results in a loss of hepatocyte microvilli (McGuire et al., 1992; Bataller and Brenner, 2005). Evidence for this is the decreased yield of membrane protein as the severity of disease increased (Figure 1). Control livers yielded the greatest amount of membrane protein, followed by HCV-cirrhotic livers and NC HCV-HCC tissues, then C HCV-HCC tissues.

In order to compensate for the differences in disease severity and membrane protein yield, transporter expression was normalized to gram of liver. In some instances this altered the pattern of transporter expression. OATP2B1, MATE1 and MRP3 expression in NC HCV-HCC tissues vs. control livers was no different when normalized to milligram of membrane protein, but significantly reduced when normalized to gram of liver (Figure 2 and 3). Adjusting for the reduced membrane protein yield amplified the difference when transporter expression in HCV-HCC tissues was lower (but not significantly different) than control livers. Changes in transporter expression were not an artefact of variation in membrane protein yield because transporter expression in some matched NC vs. C HCV-HCC tissues increased whilst others decreased (Figure 4).

Paracrine factors (e.g. hepatocyte nuclear factor 3ß, interleukin-1ß, interleukin-6 and tumour necrosis factor-α) modulate the expression of hepatobiliary drug transporters (Vavricka et al., 2004; Le Vee et al., 2008; Le Vee et al., 2009). Therefore, chronic inflammation associated with HCV-infection could be the cause of changes in transporter expression. Correspondingly, the broad inter-individual variability in transporter expression could result from variability in tissue inflammation. However, the pathophysiological role of HCV-infection as another cause cannot be disregarded. Clinically, the severity of fibrosis in

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each of our HCV-HCC samples was equal (Ishak scores: 5-6). Liver biopsies from HCV patients have shown fibrosis-dependent increases in paracrine factors and downregulation of NTCP, OATP1B1 and OCT1 mRNA-levels, with NTCP expression in the early stages of fibrosis being greater than non-infected non-cirrhotic livers (Nakai et al., 2008; Hanada et al., 2012).

Consistent with the above and our data, decreased OATP1B1, OATP1B3, OATP2B1 and OCT1 mRNA expression and OATP1B3 and OCT1 protein expression has been reported in HCV-livers vs. non-infected livers (Nakai et al., 2008; Ogasawara et al., 2010; Hanada et al., 2012; Wang et al., 2016). Decreased mRNA and protein expression of NTCP, OATP1B1, OATP1B3, OATP2B1 and OCT1 has also been reported in C HCV-HCC tissue vs. surrounding NC HCV-HCC tissue and non-infected livers (Kinoshita and Miyata, 2002; Vavricka et al., 2004; Zollner et al., 2005; Schaeffeler et al., 2011; Heise et al., 2012). Due to an inverse relationship between OATP1B1/1B3 expression and markers of HCC progression (e.g. cytokeratin polypeptided 7 and 19); OATP1B1/1B3 expression and reoccurrence-related deaths in HCV-HCC subjects; and OCT1 expression and HCC patient survival, expression of OATP1B1/B3 and OCT1 have been proposed as biomarkers of HCV-HCC (Schaeffeler et al., 2011; Vasuri et al., 2011). Decreased OATP and NTCP expression in C HCV-HCC tissue relative to NC surrounding tissue correlates with *in-vivo* data. In clinical screening, focal liver lesions are characterised by the appearance of hypointense areas created by decreased uptake of the OATP1B1, OATP1B3 and NTCP substrate Gd-EOB-DTPA (Narita et al., 2009; Tsuboyama et al., 2010).

For the expression of hepatic efflux transporters in HCV-cirrhotic vs. non-infected livers, we and others have reported (i) decreased BSEP protein expression (Wang et al., 2016) and fibrosis-dependent decreased BSEP mRNA expression (Hanada et al., 2012); (ii) increased MATE1 protein expression (Wang et al., 2016); (iii) no change (Ros et al., 2003; Nakai et al., 2008; Kurzawski et al., 2012) or decreased (Ogasawara et al., 2010; Hanada et al., 2012)

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MRP2 mRNA expression; (iv) no change in MRP3 mRNA expression (Ogasawara et al., 2010); and (v) increased P-gp mRNA expression (Ros et al., 2003; Ogasawara et al., 2010; Kurzawski et al., 2012). However, there was no change in BSEP, MRP2, MRP3 and P-gp mRNA and protein expression in C vs. surrounding NC HCV-HCC tissue (Zollner et al., 2005; Tsuboyama et al., 2010).

In summary, the majority of our results are consistent with the aforementioned published literature, but there are some discrepancies (e.g. MRP3 protein expression in HCV vs. non-infected tissue decreased rather than no change, protein expression of BSEP, MRP2 and MRP3 decreased in C vs. surrounding NC HCV-HCC tissue rather than being no different). In the above published studies the non-infected and NC HCV-HCC liver tissues were collected from patients undergoing liver resection. The sampling procedures, disease severity and treatment regimen of patients varied and could account for the observed discrepancies. Additionally, changes in mRNA expression do not always correlate with changes in protein expression (Prasad et al., 2013).

Unlike non-cirrhotic livers (r^2<0.3), a moderate correlation in hepatobiliary transporter expression was observed in NC and C HCV-HCC tissues (r^2>0.5, Supplementry Figure 1), and HCV-cirrhotic livers indicating HCV-infection directly or indirectly causes downregulation of multiple transporters (Prasad et al., 2014; Wang et al., 2015; Wang et al., 2016). No correlation was observed between transporter expression and clinical markers of liver function. However, some of the medication taken by HCV-HCC subjects (Supplementary Table 2) have been shown to alter transporter expression in animal and cell models. Therefore, drug-induced changes in transporter expression cannot be discounted.

As there were multiple differences in transporter expression between HCV-cirrhotic and NC HCV-HCC livers, we recommend that NC HCV-HCC transporter expression data (pmol/g liver) should be used in future PBPK models to predict systemic and hepatocellular drug concentrations in HCV-HCC patients. Since the volume of C in HCV-HCC is not consistent

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between HCV-HCC patients, it is much harder to individualize drug therapy based on expression of transporters in C HCV-HCC tissue. Our data suggest that there will be significant differences in local C HCV-HCC vs. NC HCV-HCC hepatic tissue drug exposure (either local C_{max} or AUC depending on the major route of elimination of the drug (Patilea-Vrana and Unadkat, 2016)). For example OCT1 expression in C HCV-HCC is approximately 20% of that in NC HCV-HCC and will therefore result in lower C_{max} of an OCT1 substrate (for example cisplatin) in C vs. NC HCV-HCC tissue. The difference, if any, in AUC in C vs NC HCV-HCC tissue will depend on whether the drug is cleared via non-hepatic clearance or not (Patilea-Vrana and Unadkat, 2016). Clinically, the volume of tumorous liver tissue in HCV-HCC subjects must be less 1% of the total liver volume for subjects to meet orthotopic liver transplantation criteria (Balogh et al., 2016).

There are some limitations to the above recommendation. First, although there were significant differences in the mean expression of many transporters between HCV cirrhotic and NC or C HCV-HCC livers, the interindividual variability was large. However, this is often the case in diseased population and therefore dosing recommendations are often based on the “average” patient. Unless biomarkers are available for all the quantified transporters, individualizing drug therapy in HCV-HCC patient will be difficult. Second, the total transporter expression in liver tissue quantified may not be functional because there may be differences in trafficking or post-translational modification caused by the disease.

In conclusion, this is the first report to quantify the abundance of major hepatobiliary drug transporters in HCV-HCC patients using quantitiative proteomics. The hepatic transporter protein expression data presented here will be useful in the development of diagnostic HCC imaging agents, drugs for the treatment of HCV-HCC and prediction of transporter-mediated drug disposition in HCV-HCC patients.

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Authorship contributions

Participated in research design: Billington, Ray, Salphati, Xiao, Chu, Humphreys, Liao, Lee, Mathias, Hop, Rowbottom, Evers, Lai, Kelly, Prasad and Unadkat.

Conducted Experiments: Billington, Kelly.

Performed Data Analysis: Billington.

Wrote or contributed in the writing of the manuscript: Billington, Ray, Salphati, Xiao, Chu, Humphreys, Liao, Lee, Mathias, Hop, Rowbottom, Evers, Lai, Kelly, Prasad and Unadkat.

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DMD Fast Forward. Published on November 14, 2017 as DOI: 10.1124/dmd.117.077289
This article has not been copyedited and formatted. The final version may differ from this version.

Reference List

Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, and Alter MJ (2006) The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* **144:**705-714.

Balogh J, Victor D, 3rd, Asham EH, Burroughs SG, Boktour M, Saharia A, Li X, Ghobrial RM, and Monsour HP, Jr. (2016) Hepatocellular carcinoma: a review. *J Hepatocell Carcinoma* **3:**41-53.

Bataller R and Brenner DA (2005) Liver fibrosis. *J Clin Invest* **115:**209-218.

Blonski W and Reddy KR (2008) Hepatitis C virus infection and hepatocellular carcinoma. *Clin Liver Dis* **12:**661-674, x.

Bonaventura A and Montecucco F (2016) Sofosbuvir/velpatasvir: A promising combination. *World J Hepatol* **8:**785-789.

Colella G, Bottelli R, De Carlis L, Sansalone CV, Rondinara GF, Alberti A, Belli LS, Gelosa F, Iamoni GM, Rampoldi A, De Gasperi A, Corti A, Mazza E, Aseni P, Meroni A, Slim AO, Finzi M, Di Benedetto F, Manochehri F, Follini ML, Ideo G, and Forti D (1998) Hepatocellular carcinoma: comparison between liver transplantation, resective surgery, ethanol injection, and chemoembolization. *Transpl Int* **11 Suppl 1:**S193-196.

Deo AK, Prasad B, Balogh L, Lai Y, and Unadkat JD (2012) Interindividual variability in hepatic expression of the multidrug resistance-associated protein 2 (MRP2/ABCC2): quantification by liquid chromatography/tandem mass spectrometry. *Drug Metab Dispos* **40:**852-855.

Desmet VJ, Gerber M, Hoofnagle JH, Manns M, and Scheuer PJ (1994) Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* **19:**1513-1520.

El-Serag HB (2012) Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* **142:**1264-1273.e1261.

Fairchild CR, Ivy SP, Kao-Shan CS, Whang-Peng J, Rosen N, Israel MA, Melera PW, Cowan KH, and Goldsmith ME (1987) Isolation of amplified and overexpressed DNA

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on November 14, 2017 as DOI: 10.1124/dmd.117.077289
This article has not been copyedited and formatted. The final version may differ from this version.

sequences from adriamycin-resistant human breast cancer cells. *Cancer Res* **47:**5141-5148.

Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, and Wong J (1999) Hepatectomy for hepatocellular carcinoma: toward zero hospital deaths. *Ann Surg* **229:**322-330.

Fong Y, Sun RL, Jarnagin W, and Blumgart LH (1999) An analysis of 412 cases of hepatocellular carcinoma at a Western center. *Ann Surg* **229:**790-799; discussion 799-800.

Freeman AJ, Dore GJ, Law MG, Thorpe M, Von Overbeck J, Lloyd AR, Marinos G, and Kaldor JM (2001) Estimating progression to cirrhosis in chronic hepatitis C virus infection. *Hepatology* **34:**809-816.

Gnoth MJ, Sandmann S, Engel K, and Radtke M (2010) In vitro to in vivo comparison of the substrate characteristics of sorafenib tosylate toward P-glycoprotein. *Drug Metab Dispos* **38:**1341-1346.

Goodgame B, Shaheen NJ, Galanko J, and El-Serag HB (2003) The risk of end stage liver disease and hepatocellular carcinoma among persons infected with hepatitis C virus: publication bias? *Am J Gastroenterol* **98:**2535-2542.

Hamm B, Staks T, Muhler A, Bollow M, Taupitz M, Frenzel T, Wolf KJ, Weinmann HJ, and Lange L (1995) Phase I clinical evaluation of Gd-EOB-DTPA as a hepatobiliary MR contrast agent: safety, pharmacokinetics, and MR imaging. *Radiology* **195:**785-792.

Hanada K, Nakai K, Tanaka H, Suzuki F, Kumada H, Ohno Y, Ozawa S, and Ogata H (2012) Effect of nuclear receptor downregulation on hepatic expression of cytochrome P450 and transporters in chronic hepatitis C in association with fibrosis development. *Drug Metab Pharmacokinet* **27:**301-306.

Hassan MM, Frome A, Patt YZ, and El-Serag HB (2002) Rising prevalence of hepatitis C virus infection among patients recently diagnosed with hepatocellular carcinoma in the United States. *J Clin Gastroenterol* **35:**266-269.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on November 14, 2017 as DOI: 10.1124/dmd.117.077289
This article has not been copyedited and formatted. The final version may differ from this version.

Hatzakis A, Wait S, Bruix J, Buti M, Carballo M, Cavaleri M, Colombo M, Delarocque-Astagneau E, Dusheiko G, Esmat G, Esteban R, Goldberg D, Gore C, Lok AS, Manns M, Marcellin P, Papatheodoridis G, Peterle A, Prati D, Piorkowsky N, Rizzetto M, Roudot-Thoraval F, Soriano V, Thomas HC, Thursz M, Valla D, van Damme P, Veldhuijzen IK, Wedemeyer H, Wiessing L, Zanetti AR, and Janssen HL (2011) The state of hepatitis B and C in Europe: report from the hepatitis B and C summit conference*. *J Viral Hepat* **18 Suppl 1:**1-16.

Heise M, Lautem A, Knapstein J, Schattenberg JM, Hoppe-Lotichius M, Foltys D, Weiler N, Zimmermann A, Schad A, Grundemann D, Otto G, Galle PR, Schuchmann M, and Zimmermann T (2012) Downregulation of organic cation transporters OCT1 (SLC22A1) and OCT3 (SLC22A3) in human hepatocellular carcinoma and their prognostic significance. *BMC Cancer* **12:**109.

Holohan C, Van Schaeybroeck S, Longley DB, and Johnston PG (2013) Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer* **13:**714-726.

Institute of Medicine Committee on the Prevention and Control of Viral Hepatitis I (2010), in: *Hepatitis and Liver Cancer: A National Strategy for Prevention and Control of Hepatitis B and C* (Colvin HM and Mitchell AE eds), National Academies Press (US)

Copyright 2010 by the National Academy of Sciences. All rights reserved., Washington (DC).

Jia J, Puls D, Oswald S, Jedlitschky G, Kuhn JP, Weitschies W, Hosten N, Siegmund W, and Keiser M (2014) Characterization of the intestinal and hepatic uptake/efflux transport of the magnetic resonance imaging contrast agent gadolinium-ethoxylbenzyl-diethylenetriamine-pentaacetic acid. *Invest Radiol* **49:**78-86.

Johnston RA, Rawling T, Chan T, Zhou F, and Murray M (2014) Selective inhibition of human solute carrier transporters by multikinase inhibitors. *Drug Metab Dispos* **42:**1851-1857.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on November 14, 2017 as DOI: 10.1124/dmd.117.077289
This article has not been copyedited and formatted. The final version may differ from this version.

Kalliokoski A, Backman JT, Neuvonen PJ, and Niemi M (2008) Effects of the SLCO1B1*1B haplotype on the pharmacokinetics and pharmacodynamics of repaglinide and nateglinide. *Pharmacogenet Genomics* **18:**937-942.

Kamiie J, Ohtsuki S, Iwase R, Ohmine K, Katsukura Y, Yanai K, Sekine Y, Uchida Y, Ito S, and Terasaki T (2008) Quantitative atlas of membrane transporter proteins: development and application of a highly sensitive simultaneous LC/MS/MS method combined with novel in-silico peptide selection criteria. *Pharm Res* **25:**1469-1483.

Kinoshita M and Miyata M (2002) Underexpression of mRNA in human hepatocellular carcinoma focusing on eight loci. *Hepatology* **36:**433-438.

Kitao A, Zen Y, Matsui O, Gabata T, Kobayashi S, Koda W, Kozaka K, Yoneda N, Yamashita T, Kaneko S, and Nakanuma Y (2010) Hepatocellular carcinoma: signal intensity at gadoxetic acid-enhanced MR Imaging--correlation with molecular transporters and histopathologic features. *Radiology* **256:**817-826.

Kurzawski M, Dziedziejko V, Post M, Wojcicki M, Urasinska E, Mietkiewski J, and Drozdzik M (2012) Expression of genes involved in xenobiotic metabolism and transport in end-stage liver disease: up-regulation of ABCC4 and CYP1B1. *Pharmacol Rep* **64:**927-939.

Lagas JS, van Waterschoot RA, Sparidans RW, Wagenaar E, Beijnen JH, and Schinkel AH (2010) Breast cancer resistance protein and P-glycoprotein limit sorafenib brain accumulation. *Mol Cancer Ther* **9:**319-326.

Le Vee M, Gripon P, Stieger B, and Fardel O (2008) Down-regulation of organic anion transporter expression in human hepatocytes exposed to the proinflammatory cytokine interleukin 1beta. *Drug Metab Dispos* **36:**217-222.

Le Vee M, Lecureur V, Stieger B, and Fardel O (2009) Regulation of drug transporter expression in human hepatocytes exposed to the proinflammatory cytokines tumor necrosis factor-alpha or interleukin-6. *Drug Metab Dispos* **37:**685-693.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on November 14, 2017 as DOI: 10.1124/dmd.117.077289
This article has not been copyedited and formatted. The final version may differ from this version.

Leonhardt M, Keiser M, Oswald S, Kuhn J, Jia J, Grube M, Kroemer HK, Siegmund W, and Weitschies W (2010) Hepatic uptake of the magnetic resonance imaging contrast agent Gd-EOB-DTPA: role of human organic anion transporters. *Drug Metab Dispos* **38:**1024-1028.

Lok AS, Seeff LB, Morgan TR, di Bisceglie AM, Sterling RK, Curto TM, Everson GT, Lindsay KL, Lee WM, Bonkovsky HL, Dienstag JL, Ghany MG, Morishima C, and Goodman ZD (2009) Incidence of hepatocellular carcinoma and associated risk factors in hepatitis C-related advanced liver disease. *Gastroenterology* **136:**138-148.

Lozano R Naghavi M Foreman K Lim S Shibuya K Aboyans V Abraham J Adair T Aggarwal R Ahn SY Alvarado M Anderson HR Anderson LM Andrews KG Atkinson C Baddour LM Barker-Collo S Bartels DH Bell ML Benjamin EJ Bennett D Bhalla K Bikbov B Bin Abdulhak A Birbeck G Blyth F Bolliger I Boufous S Bucello C Burch M Burney P Carapetis J Chen H Chou D Chugh SS Coffeng LE Colan SD Colquhoun S Colson KE Condon J Connor MD Cooper LT Corriere M Cortinovis M de Vaccaro KC Couser W Cowie BC Criqui MH Cross M Dabhadkar KC Dahodwala N De Leo D Degenhardt L Delossantos A Denenberg J Des Jarlais DC Dharmaratne SD Dorsey ER Driscoll T Duber H Ebel B Erwin PJ Espindola P Ezzati M Feigin V Flaxman AD Forouzanfar MH Fowkes FG Franklin R Fransen M Freeman MK Gabriel SE Gakidou E Gaspari F Gillum RF Gonzalez-Medina D Halasa YA Haring D Harrison JE Havmoeller R Hay RJ Hoen B Hotez PJ Hoy D Jacobsen KH James SL Jasrasaria R Jayaraman S Johns N Karthikeyan G Kassebaum N Keren A Khoo JP Knowlton LM Kobusingye O Koranteng A Krishnamurthi R Lipnick M Lipshultz SE Ohno SL Mabweijano J MacIntyre MF Mallinger L March L Marks GB Marks R Matsumori A Matzopoulos R Mayosi BM McAnulty JH McDermott MM McGrath J Mensah GA Merriman TR Michaud C Miller M Miller TR Mock C Mocumbi AO Mokdad AA Moran A Mulholland K Nair MN Naldi L Narayan KM Nasseri K Norman P O'Donnell M Omer SB Ortblad K Osborne R Ozgediz D Pahari B Pandian JD Rivero AP Padilla RP Perez-Ruiz F

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Perico N Phillips D Pierce K Pope CA, 3rd Porrini E Pourmalek F Raju M Ranganathan D Rehm JT Rein DB Remuzzi G Rivara FP Roberts T De Leon FR Rosenfeld LC Rushton L Sacco RL Salomon JA Sampson U Sanman E Schwebel DC Segui-Gomez M Shepard DS Singh D Singleton J Sliwa K Smith E Steer A Taylor JA Thomas B Tleyjeh IM Towbin JA Truelsen T Undurraga EA Venketasubramanian N Vijayakumar L Vos T Wagner GR Wang M Wang W Watt K Weinstock MA Weintraub R Wilkinson JD Woolf AD Wulf S Yeh PH Yip P Zabetian A Zheng ZJ Lopez AD Murray CJ AlMazroa MA and Memish ZA (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* **380:**2095-2128.

McGuire RF, Bissell DM, Boyles J, and Roll FJ (1992) Role of extracellular matrix in regulating fenestrations of sinusoidal endothelial cells isolated from normal rat liver. *Hepatology* **15:**989-997.

Morgan RL, Baack B, Smith BD, Yartel A, Pitasi M, and Falck-Ytter Y (2013) Eradication of hepatitis C virus infection and the development of hepatocellular carcinoma: a meta-analysis of observational studies. *Ann Intern Med* **158:**329-337.

Nakai K, Tanaka H, Hanada K, Ogata H, Suzuki F, Kumada H, Miyajima A, Ishida S, Sunouchi M, Habano W, Kamikawa Y, Kubota K, Kita J, Ozawa S, and Ohno Y (2008) Decreased expression of cytochromes P450 1A2, 2E1, and 3A4 and drug transporters Na+-taurocholate-cotransporting polypeptide, organic cation transporter 1, and organic anion-transporting peptide-C correlates with the progression of liver fibrosis in chronic hepatitis C patients. *Drug Metab Dispos* **36:**1786-1793.

Narita M, Hatano E, Arizono S, Miyagawa-Hayashino A, Isoda H, Kitamura K, Taura K, Yasuchika K, Nitta T, Ikai I, and Uemoto S (2009) Expression of OATP1B3 determines uptake of Gd-EOB-DTPA in hepatocellular carcinoma. *J Gastroenterol* **44:**793-798.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on November 14, 2017 as DOI: 10.1124/dmd.117.077289
This article has not been copyedited and formatted. The final version may differ from this version.

Nies AT, Niemi M, Burk O, Winter S, Zanger UM, Stieger B, Schwab M, and Schaeffeler E (2013) Genetics is a major determinant of expression of the human hepatic uptake transporter OATP1B1, but not of OATP1B3 and OATP2B1. *Genome Med* **5:**1.

Ogasawara K, Terada T, Katsura T, Hatano E, Ikai I, Yamaoka Y, and Inui K (2010) Hepatitis C virus-related cirrhosis is a major determinant of the expression levels of hepatic drug transporters. *Drug Metab Pharmacokinet* **25:**190-199.

Patilea-Vrana G and Unadkat JD (2016) Transport vs. Metabolism: What Determines the Pharmacokinetics and Pharmacodynamics of Drugs? Insights From the Extended Clearance Model. *Clin Pharmacol Ther* **100:**413-418.

Prasad B, Evers R, Gupta A, Hop CE, Salphati L, Shukla S, Ambudkar SV, and Unadkat JD (2014) Interindividual variability in hepatic organic anion-transporting polypeptides and P-glycoprotein (ABCB1) protein expression: quantification by liquid chromatography tandem mass spectroscopy and influence of genotype, age, and sex. *Drug Metab Dispos* **42:**78-88.

Prasad B, Gaedigk A, Vrana M, Gaedigk R, Leeder JS, Salphati L, Chu X, Xiao G, Hop C, Evers R, Gan L, and Unadkat JD (2016) Ontogeny of Hepatic Drug Transporters as Quantified by LC-MS/MS Proteomics. *Clin Pharmacol Ther* **100:**362-370.

Prasad B, Lai Y, Lin Y, and Unadkat JD (2013) Interindividual variability in the hepatic expression of the human breast cancer resistance protein (BCRP/ABCG2): effect of age, sex, and genotype. *J Pharm Sci* **102:**787-793.

Prasad B and Unadkat JD (2014) Optimized approaches for quantification of drug transporters in tissues and cells by MRM proteomics. *Aaps j* **16:**634-648.

Rantala M and van de Laar MJ (2008) Surveillance and epidemiology of hepatitis B and C in Europe - a review. *Euro Surveill* **13**.

Ros JE, Libbrecht L, Geuken M, Jansen PL, and Roskams TA (2003) High expression of MDR1, MRP1, and MRP3 in the hepatic progenitor cell compartment and hepatocytes in severe human liver disease. *J Pathol* **200:**553-560.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on November 14, 2017 as DOI: 10.1124/dmd.117.077289
This article has not been copyedited and formatted. The final version may differ from this version.

Saeed M, Kadioglu O, Khalid H, Sugimoto Y, and Efferth T (2015) Activity of the dietary flavonoid, apigenin, against multidrug-resistant tumor cells as determined by pharmacogenomics and molecular docking. *J Nutr Biochem* **26:**44-56.

Schaeffeler E, Hellerbrand C, Nies AT, Winter S, Kruck S, Hofmann U, van der Kuip H, Zanger UM, Koepsell H, and Schwab M (2011) DNA methylation is associated with downregulation of the organic cation transporter OCT1 (SLC22A1) in human hepatocellular carcinoma. *Genome Med* **3:**82.

Schuhmann-Giampieri G, Schmitt-Willich H, Press WR, Negishi C, Weinmann HJ, and Speck U (1992) Preclinical evaluation of Gd-EOB-DTPA as a contrast agent in MR imaging of the hepatobiliary system. *Radiology* **183:**59-64.

Swift B, Nebot N, Lee JK, Han T, Proctor WR, Thakker DR, Lang D, Radtke M, Gnoth MJ, and Brouwer KL (2013) Sorafenib hepatobiliary disposition: mechanisms of hepatic uptake and disposition of generated metabolites. *Drug Metab Dispos* **41:**1179-1186.

Tariq M, Alam MA, Singh AT, Panda AK, and Talegaonkar S (2016) Surface decorated nanoparticles as surrogate carriers for improved transport and absorption of epirubicin across the gastrointestinal tract: Pharmacokinetic and pharmacodynamic investigations. *Int J Pharm* **501:**18-31.

Terrault NA, Zeuzem S, Di Bisceglie AM, Lim JK, Pockros PJ, Frazier LM, Kuo A, Lok AS, Shiffman ML, Ben Ari Z, Akushevich L, Vainorius M, Sulkowski MS, Fried MW, and Nelson DR (2016) Effectiveness of Ledipasvir-Sofosbuvir Combination in Patients With Hepatitis C Virus Infection and Factors Associated With Sustained Virologic Response. *Gastroenterology* **151:**1131-1140.e1135.

Tsuboyama T, Onishi H, Kim T, Akita H, Hori M, Tatsumi M, Nakamoto A, Nagano H, Matsuura N, Wakasa K, and Tomoda K (2010) Hepatocellular carcinoma: hepatocyte-selective enhancement at gadoxetic acid-enhanced MR imaging--correlation with expression of sinusoidal and canalicular transporters and bile accumulation. *Radiology* **255:**824-833.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 20, 2024

DMD Fast Forward. Published on November 14, 2017 as DOI: 10.1124/dmd.117.077289
This article has not been copyedited and formatted. The final version may differ from this version.

Vasuri F, Golfieri R, Fiorentino M, Capizzi E, Renzulli M, Pinna AD, Grigioni WF, and D'Errico-Grigioni A (2011) OATP 1B1/1B3 expression in hepatocellular carcinomas treated with orthotopic liver transplantation. *Virchows Arch* **459:**141-146.

Vavricka SR, Jung D, Fried M, Grutzner U, Meier PJ, and Kullak-Ublick GA (2004) The human organic anion transporting polypeptide 8 (SLCO1B3) gene is transcriptionally repressed by hepatocyte nuclear factor 3beta in hepatocellular carcinoma. *J Hepatol* **40:**212-218.

Wang L, Collins C, Kelly EJ, Chu X, Ray AS, Salphati L, Xiao G, Lee C, Lai Y, Liao M, Mathias A, Evers R, Humphreys W, Hop CE, Kumer SC, and Unadkat JD (2016) Transporter Expression in Liver Tissue from Subjects with Alcoholic or Hepatitis C Cirrhosis Quantified by Targeted Quantitative Proteomics. *Drug Metab Dispos* **44:**1752-1758.

Wang L, Prasad B, Salphati L, Chu X, Gupta A, Hop CE, Evers R, and Unadkat JD (2015) Interspecies variability in expression of hepatobiliary transporters across human, dog, monkey, and rat as determined by quantitative proteomics. *Drug Metab Dispos* **43:**367-374.

Wei Y, Ma Y, Zhao Q, Ren Z, Li Y, Hou T, and Peng H (2012) New use for an old drug: inhibiting ABCG2 with sorafenib. *Mol Cancer Ther* **11:**1693-1702.

World Health Organisation (2017) Global Hepatitis Report 2017 (Geneva: World Health Organisation ed.

Yonezawa A, Masuda S, Yokoo S, Katsura T, and Inui K (2006) Cisplatin and oxaliplatin, but not carboplatin and nedaplatin, are substrates for human organic cation transporters (SLC22A1-3 and multidrug and toxin extrusion family). *J Pharmacol Exp Ther* **319:**879-886.

Zimmerman EI, Hu S, Roberts JL, Gibson AA, Orwick SJ, Li L, Sparreboom A, and Baker SD (2013) Contribution of OATP1B1 and OATP1B3 to the disposition of sorafenib and sorafenib-glucuronide. *Clin Cancer Res* **19:**1458-1466.

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Zollner G, Wagner M, Fickert P, Silbert D, Fuchsbichler A, Zatloukal K, Denk H, and Trauner M (2005) Hepatobiliary transporter expression in human hepatocellular carcinoma. *Liver Int* **25:**367-379.

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Footnotes

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Figure Legends

Figure 1: Total membrane protein yield from (A) control livers, HCV-cirrhotic livers, NC HCV-HCC and C HCV-HCC liver tissues, or (B) matched NC HCV-HCC and C HCV-HCC liver tissues. (A) The membrane protein yield from HCV-cirrhotic livers, NC and C HCV-HCC tissues was significantly lower than that from the control livers. The total membrane protein yield from C HCV-HCC tissues was also significantly lower than NC HCV-HCC tissues. Data on the total membrane protein yield of control and HCV-cirrhotic livers were taken from Prasad et al (2016) and Wang et al. (2016), respectively. The data are represented as box-plots the median (horizontal line), 75^{th} (top of box) and 25^{th} (bottom of box) quartiles, the smallest and largest values (whiskers) and mean (+) are shown. The symbols 'a', 'b', and 'c' indicate a significant difference from control livers, HCV-cirrhotic livers, NC HCV-HCC tissues, respectively ($p < 0.05$, Kruskal-Wallis test). (B) In matched tissues, the yield of membrane protein from C HCV-HCC tissues was lower, but not significantly different ($p=0.055$, Wilcoxon test), than that from NC-HCV-HCC tissues (n=8). Each line connects data from the matched liver samples.

Figure 2: Protein expression of sinusoidal uptake transporters in control livers, HCV-cirrhotic livers, NC HCV-HCC and C HCV-HCC liver tissues normalized to milligram of membrane protein (upper panel) or gram of liver (lower panel). In comparison to control livers (per gram of liver), except for NTCP, transporter protein expression was reduced in both NC and C HCV-HCC tissues. NTCP expression was increased in NC HCV-HCC tissues and decreased in C HCV-HCC tissues relative to control livers. Similarly, compared to HCV-cirrhotic livers (per gram of liver), OATP1B1, OATP1B3, and OATP2B1 protein expression was decreased in both NC and C HCV-HCC tissues. NTCP protein expression in NC HCV-HCC tissues was greater than HCV-cirrhotic livers. OCT1 expression in C HCV-HCC tissues was lower than HCV-cirrhotic livers. Transporter expression data for control and HCV-cirrhotic livers were taken from Prasad et al (2016) and Wang et al. (2016), respectively. The data are represented as box-plots the median

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(horizontal line), 75th (top of box) and 25th (bottom of box) quartiles, the smallest and largest values (whiskers) and mean (+) are shown. The symbols 'a', 'b', and 'c' indicate a significant difference from control livers, HCV-cirrhotic livers, NC HCV-HCC liver tissues, respectively ($p < 0.05$, Kruskal-Wallis test).

Figure 3: The protein expression of sinusoidal and canalicular efflux transporters in control livers, HCV-cirrhotic livers, NC HCV-HCC and C HCV-HCC liver tissues normalized to milligram of membrane protein (upper panel) or gram of liver (lower panel). In comparison to control livers (per gram of liver), except for MATE1 and MRP2, transporter protein expression decreased in both NC and C HCV-HCC tissues. MATE1 expression in NC HCV-HCC tissues and MRP2 in C HCV-HCC tissues were lower than control livers. Likewise, generally transporter expression in both NC and C HCV-HCC tissues were lower than HCV-cirrhotic livers (per gram of liver). The exceptions being, BSEP expression was no different and MRP2 expression increased in NC HCV-HCC tissues, and MRP2 expression was no different in C HCV-HCC tissues from HCV-cirrhotic livers. Transporter expression data for control and HCV-cirrhotic livers were taken from Prasad et al (2016) and Wang et al. (2016), respectively. The data are represented as box-plots the median (horizontal line), 75th (top of box) and 25th (bottom of box) quartiles, the smallest and largest values (whiskers) and mean (+) are shown. The symbols 'a', 'b', and 'c' indicate a significant difference from control livers, HCV-cirrhotic livers, NC HCV-HCC tissues, respectively ($p < 0.05$, Kruskal-Wallis test).

Figure 4: Transport protein expression (per gram of liver) in matched NC and C HCV-HCC liver tissues. Expression of OATP2B1, BSEP, MRP2 and MRP3 were significantly lower in C HCV-HCC tissues than NC HCV-HCC tissues. Expression of NTCP and OCT1 in C tissues also showed a tendency towards significance. The symbol * indicates a statistical significance ($p<0.05$, Wilcoxon test. Each line connects data from the matched liver samples.

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Table 1: Demographics of control, HCV-cirrhotic, and HCV-infected NC and C livers. The normal ranges for these covariates are the following; BMI: 18.5 to 24.9, Creatinine: 0.5 to 1.5 mg/dL, GFR: 90 to 120 mL/min, INR: 0.8 to 1.2, Alkaline Phosphatase: 44 to 147 U/L, Alanine Aminotransferase: 7 to 40 U/L, Aspartate Aminotransferase: 10 to 34 U/L, Albumin: 3.5 to 5.5 g/dL, Total Bilirubin: 0.3 to 1.9 mg/dL. Data represented as the mean ± SD.

	Control	HCV-Cirrhotic	HCV-HCC (Gilead)	HCV-HCC (*LTCDS)
Sample Size	36	30	25	8
Age (years)	47 ± 14	53 ± 8	59 ± 5	59 ± 6
Sex	18 Male, 18 Female	18 Male, 12 Female	20 Male, 5 Female	7 Male, 1 Female
Race	33 Caucasian, 2 African American, 1 Asian	24 Caucasian, 4 African American, 1 Hispanic	23 Caucasian, 1 African American, 1 Hispanic	5 Caucasian, 2 African American, 1 Hispanic
BMI	NR	31.7 ± 6.3	29.2 ± 7.6	30.4 ± 6.5
Creatinine (mg/dL)	NR	NR	NR	1.0 ± 0.5
GFR (Cockcroft-Gault, mL/min)	NR	NR	126 ± 30	88 ± 28
INR	NR	1.7 ± 0.5	NR	1.4 ± 0.7
Alkaline Phosphatase (U/L)	NR	112 ± 54	NR	133 ± 49
Alanine Aminotransferase (U/L)	NR	146 ± 243	77 ± 38	82 ± 104
Aspartate Aminotransferase (U/L)	NR	223 ± 388	NR	87 ± 74
Albumin (g/dL)	NR	2.6 ± 0.6	NR	3.3 ± 0.5
Total bilirubin (mg/dL)	NR	3.6 ± 2.9	NR	1.2 ± 0.7
Treatment Regimen	NR	NR	25 SOF + RBV	3 SOF + RBV, 1 SOF + RBV+ PEG-IFN, 1 LDV, 3 NR

*LTCDS: Available as matched non-cancerous and cancerous HCV-HCC liver samples.
Abbreviations - U: Units, SOF: Sofosbuvir, RBV: Ribavarin, PEG-IFN: Peginterferon alpha-2a, LDV: Ledipasvir, NR: Not recorded.

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Figure 1

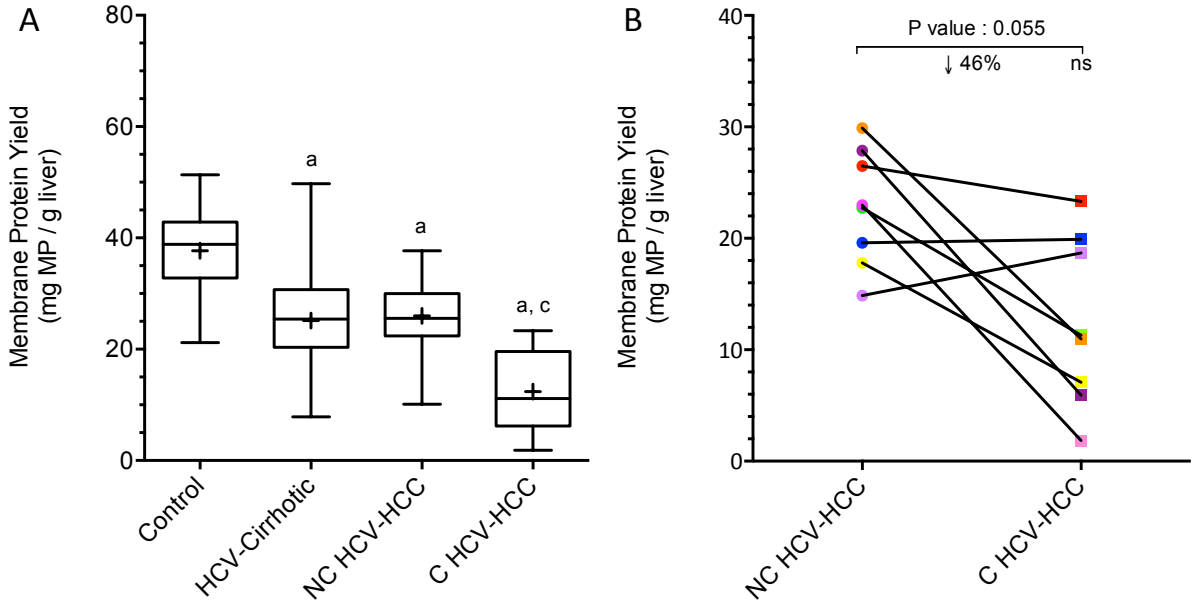

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Figure 2

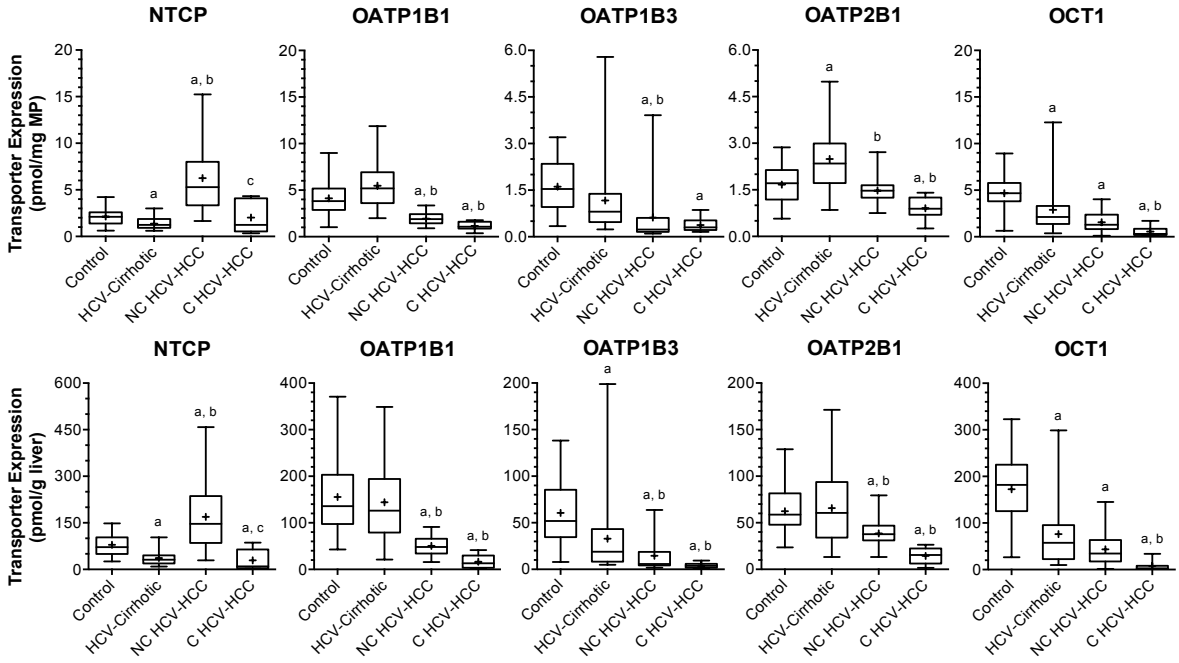

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Figure 3

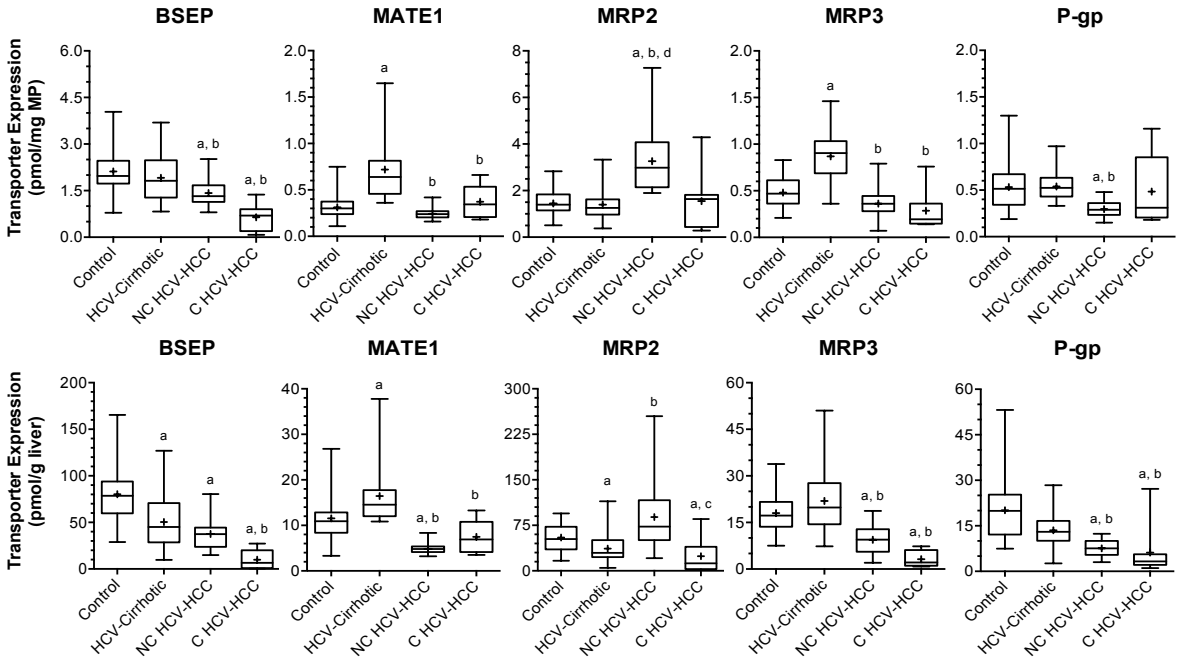

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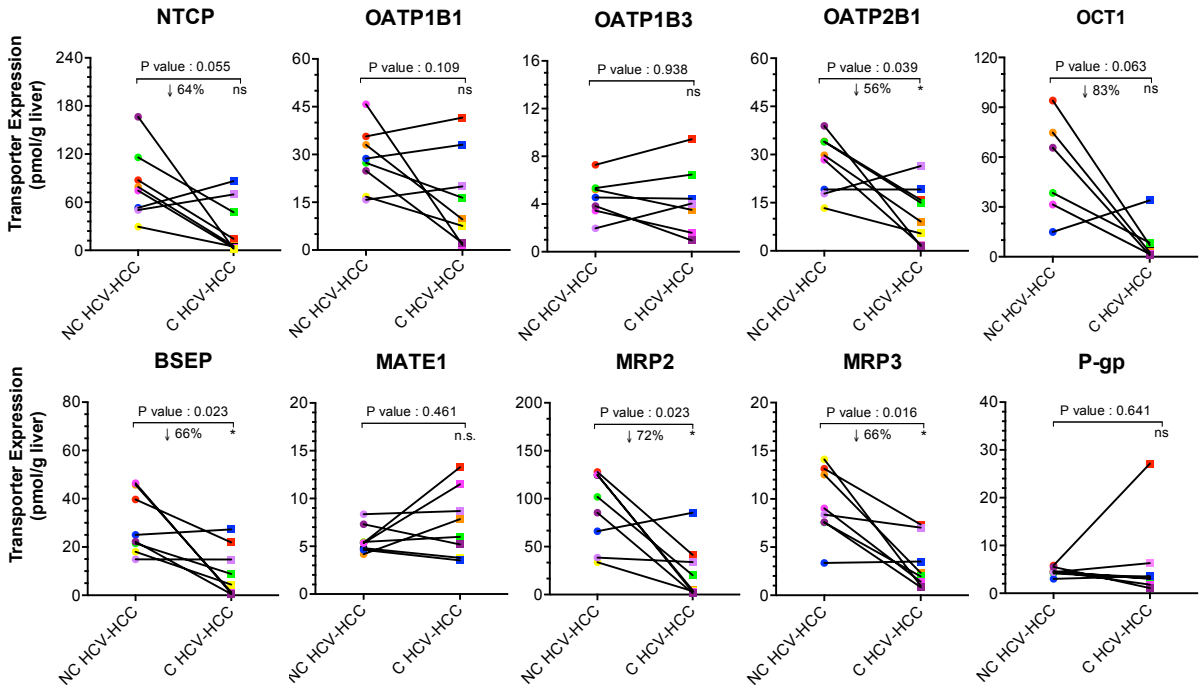
Figure 4



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