The liver plays an essential role in removing endogenous and exogenous compounds from the circulation. This function is mediated by specific transporters, including members of the family of organic anion transport proteins (OATPs) and the Na\(^+\)-taurocholate transporting polypeptide (NTCP). In the present study, transporter protein expression was determined in liver samples from patients with cirrhosis or controls without liver disease. Five transporters (OATP1A2, OATP1B1, OATP1B3, OATP2B1, and NTCP) were studied. Transporter content in homogenates of human liver was quantified on western blots probed with transporter-specific antibodies in which a calibrated green fluorescent protein-tagged transporter standard was included. Liver samples from 21 patients with cirrhosis (hepatitis C in 17 and alcohol abuse in 4) and 17 controls without liver disease were analyzed. Expression of each of the transporters had a large spread, varying by an order of magnitude in cirrhotic and control livers. OATP1B1 was the most abundant transporter in controls (\(P < 0.01\)) but was significantly lower in cirrhotic livers as was NTCP expression (\(P < 0.01\)). There was little difference in transporter expression with respect to age or sex. Despite the large variability in transporter expression within a group, analysis in individuals showed that those with high or low expression of one transporter had a similar magnitude in expression of the others. Conclusion: Differences in transporter expression could explain unanticipated heterogeneity of drug transport and metabolism in individuals with and without liver disease. (Hepatology Communications 2020;4:739-752).
have been associated with reduced plasma clearance of statins with corresponding increased incidence of myopathy and reduced efficacy.\(^{(12,13)}\)

These transporters have significant overlap in their substrate specificities, and it has been difficult to predict transported ligands based on their structure, although pharmacophore modeling has been helpful in this regard.\(^{(14)}\) There is also significant heterogeneity in drug transport among individuals. Several previous studies looked for heterogeneous expression of specific transporters in human liver by western blot\(^{(15)}\) or liquid chromatography–mass spectrometry (LC-MS).\(^{(16-19)}\) Although these studies provided important insights, there were differences in transporter expression between studies that were likely attributable to variations in sample preparation and methodology.\(^{(17,20)}\) To obviate some of these concerns, the present investigation, using tagged transporter standards and immunologic detection, provides an alternative complementary procedure for direct comparison of transporter expression in human liver homogenates. Importantly, this analysis requires minimal sample preparation and manipulation.

In the present study, expression of key organic anion transporters (OATP1A2, OATP1B1, OATP1B3, OATP2B1, and NTCP) was quantified in human liver samples that were obtained from a cohort of individuals with and without cirrhosis. These studies employed specific antibodies to detect transporters and green fluorescent protein (GFP)-tagged transporter standards. These procedures permit the comparison of expression of these transporters within a single patient’s liver as well as comparison of transporters between patients. Although expression of these transporters may be altered with disease, it is recognized that changes in drug transport in liver disease, including cirrhosis, may be multifactorial, including altered vascular architecture of the liver in addition to altered expression of specific transporters.\(^{(3,21)}\) The influence of factors, such as age, sex, and ethnicity, on transporter expression in cirrhotic and control liver was also examined.

**Materials and Methods**

**PREPARATION OF GFP-TAGGED TRANSPORTER EXPRESSION PLASMIDS**

A superfolder GFP (sfGFP) encoding plasmid\(^{(22)}\) was provided by the Imaging and Cell Structure Core of the Marion Bessin Liver Research Center at the Albert Einstein College of Medicine, Bronx, NY. Expression plasmids encoding human OATP1B1 (pEFT6-LSI-OATP1B1), OATP1A2 (pCDNA3.1-Zeo-OATP1A2), and NTCP (pGEX6p-1-NTCP) were a gift from Dr. Richard Kim at Western University, London, Canada. Expression plasmids encoding OATP1B3 (pCMV6-OATP1B3) and OATP2B1 (pCMV-OATP2B1) were a gift from Dr. I. David Goldman of the Albert Einstein College of Medicine. Complementary DNAs (cDNAs) for insertion into the sfGFP plasmid were prepared from these plasmids by polymerase chain reaction using the following primers: OATP1A2, sense 5′CGGGGTACCATGGGAGAAACTGAGAAAAGAATTGAAACCC3′, antisense 5′CCGCTCGAGTTACAAATTTGAATAAAACA3′; OATP1B1, sense 5′CCGCCTGAGATTACAATTTTAGTTTTCAATTCACTCATTACC3′; OATP1B2, sense 5′CCGCTGAGATTACAATTTTAGTTTTCAATTCACTCATTACC3′; OATP1B3, sense 5′CCGCCTGAGATTACAATTTTAGTTTTCAATTCACTCATTACC3′; OATP2B1, sense 5′CCGCCTGAGATTACAATTTTAGTTTTCAATTCACTCATTACC3′; OATP2B2, sense 5′CCGCCTGAGATTACAATTTTAGTTTTCAATTCACTCATTACC3′; OATP2B3, sense 5′CCGCCTGAGATTACAATTTTAGTTTTCAATTCACTCATTACC3′; NTCP, sense 5′CCGCCTGAGATTACAATTTTAGTTTTCAATTCACTCATTACC3′; antisense 5′CCGCTGAGATTACAATTTTAGTTTTCAATTCACTCATTACC3′.
CCAGCA3'; OATP1B3, sense 5'CCGCTCGAGGATCCATGGGAC
GGAGTACCAGCGCCGGGT3', antisense 5'CC
CAAGCTTACACTGGAATCTCCTG
GCTTTCTCCCTGGCC3'; NTCP, sense CC
AGTTCGATGGGAGCCTCAAACGC
GCTGCCCCATT; antisense CGCGGATCCCTA
GCCGACGTCGACTTAGTTGGCAGCAG
CATTGTCTTG CATGTC3'.

cDNAs were inserted into the sfGFP plasmid using the following restriction sites: KpnI and XbaI for OATP1A2, XhoI and KpnI for OATP1B1, XhoI and SalI for OATP1B3, XhoI and HindIII for OATP2B1, and HindIII and BamH1 for NTCP.

Each of the sfGFP-transporter plasmids was transiently transfected into HEK293FT cells (Thermo Fisher, Waltham, MA) for 48 hours. In brief, cells were maintained in 10-cm polystyrene culture dishes (Corning #430167) in Dulbecco's modified Eagle's medium containing 4.5 g/L glucose, L-glutamine, and sodium pyruvate (Mediatech, Manassas, VA). All media were supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA) and 1% penicillin–streptomycin (Mediatech). Plasmid DNA (20 µg) in 450 µL of water was vortexed for 30 seconds with 50 µL of 2.5 M CaCl2 and 500 µL of 2× 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES) buffered saline (100 mM HEPES, 280 mM NaCl, and 1.5 mM Na2HPO4, adjusted to pH 7.12 with NaOH) and let stand for 10 minutes at room temperature. The 1-mL mixture was then added to one culture dish containing HEK293FT cells at approximately 70%-80% confluence in 8 mL medium. Following an overnight incubation at 37°C in 5% CO2, the culture medium was replaced with fresh medium. Twenty-four hours later, cells were washed 3 times with phosphate-buffered saline (PBS) and lysed in PBS containing 1 mM ethylene diamine tetraacetic acid and a protease inhibitor cocktail (cOmplete mini; Sigma-Aldrich, St. Louis, MO) and 1% Triton X-100. The lysate was clarified by centrifugation at 20,800g at 4°C, and reactivity with antibody to GFP as well as antibody to the specific transporter was determined by immunoblot.

HUMAN LIVER TISSUE

Thirty-eight liver samples, 21 from patients with morphologically proven cirrhosis and 17 from control patients without liver disease, were obtained from our institutional review board-approved biorepository. Samples of cirrhotic liver were obtained from liver explants at the time of transplantation (14 patients) or liver resections (7 patients), while control liver specimens were obtained from normal-appearing liver during surgical resection procedures for metastatic colon cancer (16 patients) or cyst resection (1 patient). Samples were placed on ice soon after resection and cleared by pathology for inclusion in the biorepository, generally within 30 minutes. Basic demographic information, including age, sex, and ethnicity, as well as blood chemistry results are summarized in Table 1. Liver samples were homogenized using a Bullet Blender Homogenizer (Next Advance, Inc., Troy, NY). Briefly, approximately 40 mg liver tissue in 1.0 mL PBS containing protease inhibitor cocktail was homogenized with 750 µg of 1.0-mm Zirconium Oxide Beads (Next Advance, Inc.) at a setting of 3 for 3 minutes and then at a setting of 12 for 5 minutes at 4°C. The homogenate was centrifuged at 350g for 6 minutes at 4°C, and the supernatant was stored at −80°C until used. Total protein concentration was determined using the Pierce bicinchoninic acid (BCA) kit (Pierce Biotechnology, Rockford, IL) with bovine serum albumin as the standard.

| TABLE 1. PATIENT CHARACTERISTICS |
|----------------------------------|
|                                | Control (n = 17) | Cirrhosis (n = 21): 17 HCV, 4 EtOH |
| Age (years)                    | 56 ± 12         | 58 ± 7                     |
| Range                        | 32-75           | 41-72                      |
| Sex                           | 7 M; 10 F       | 10 M; 11 F                 |
| Ethnicity                     | 7 AA; 6 H; 4 C  | 5 AA; 8 H; 3 C; 5 U       |
| BMI                           | 26.9 ± 4.8      | 32.0 ± 8.6                 |
| INR                           | 1.1 ± 0.1       | 1.6 ± 0.5*                 |
| Alkaline phosphatase (IU) (nL <130) | 105.8 ± 34.2        | 147.2 ± 121.5*            |
| Alanine aminotransferase (IU) (nL <30) | 23.3 ± 10.4       | 55.0 ± 32.2*              |
| Aspartate aminotransferase (IU) (nL <40) | 26.9 ± 14.8     | 80.6 ± 42*                |
| Albumin (g/dL) (nL >3.5)       | 4.2 ± 0.4       | 3.4 ± 0.6*                 |
| Total bilirubin (mg/dL) (nL <1.2) | 0.6 ± 0.5       | 7.1 ± 7.8*                 |

*P < 0.01 compared to control.
Abbreviations: AA, African American; BMI, body mass index; C, Caucasian; EtOH, ethanol; F, female; H, Hispanic; HCV, hepatitis C virus; INR, international normalized ratio; IU, international unit; M, male; U, Unknown.
WESTERN BLOT ANALYSIS

Western blot was performed as described.\(^{25}\) In brief, tissue lysates (30 µg protein) were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (100 mM dithiothreitol) and transferred to a polyvinylidene difluoride (PVDF) membrane (Perkin Elmer, Boston, MA). The PVDF membrane was blocked with 10% nonfat dry milk in trishydroxymethylaminomethane-buffered saline (TBS)-Tween (50 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.6) prior to incubation with primary antibody diluted with 2% nonfat dry milk in TBS-Tween. The following rabbit polyclonal antibodies were used: anti-GFP (NB600-308; Novus Biologicals LLC, Littleton, CO), anti-OATP1A2 (NBP1-59658; Novus Biologicals), anti-OATP1B1 prepared as described,\(^{26}\) anti-OATP1B3 (OAAB14502; Aviva Systems Biology Co., San Diego, CA), anti-OATP2B1 (NBP1-80979; Novus Biologicals), and anti-NTCP (NBP1-92393; Novus Biologicals). Immunoblot analysis was performed using appropriate horseradish peroxidase-conjugated secondary antibody (Jackson Immuno Research, West Grove, PA). Bands were detected and intensity was quantified using an image analyzer FluorChem Q (Protein Simple, San Jose, CA).

QUANTIFICATION OF TRANSPORTERS IN HUMAN LIVER SAMPLES

Western blots of lysates from transfected HEK293FT cells expressing sfGFP-transporter fusion proteins were performed using antibody to GFP (Fig. 1A). As there is one GFP molecule linked to one transporter molecule, the GFP content in each cell lysate is proportional to the concentration of transporter in that lysate. For example, equal GFP expression on western blot for each of the cell lysates would imply equal concentrations of transporters in those lysates. The total protein for each HEK293FT cell lysate that was analyzed as a standard in every

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**FIG. 1.** Determination of transporter protein expression in human liver lysates. (A) GFP-fusion proteins of each of the five transporters that were studied were prepared in HEK293FT cells, as described in Materials and Methods. SDS-PAGE of aliquots of each of the HEK293FT lysates was performed, transferred to PVDF membrane, and subjected to western blot using GFP antibody. In this representative experiment of three that were performed, the amount (µg) of cell lysate protein applied to the gel was adjusted to provide similar intensities for each of the GFP-transporter fusion proteins, as indicated. Relative expression of GFP-OATPs was obtained by densitometry, and the means ± SD of the three experiments are indicated in the figure. (B) In these representative western blots, lysates of liver samples from 3 patients as well as appropriate calibrated HEK293FT lysates containing approximately equal amounts of GFP-transporter fusion proteins were subjected to SDS-PAGE followed by western blots, using specific transporter antibodies as indicated.
immunoblot and the relative expression (RE) of the corresponding GFP-transporter protein is shown in Fig. 1A as means ± SD of triplicate assays. These proteins were then used as calibration standards when run on western blots along with human liver lysates and probed with transporter-specific antibody (Fig. 1B). Transporter content (GFP units) was calculated as follows:

\[
\text{GFP Unit} = \frac{\text{Intensity of liver transporter band}}{\text{Intensity of GFP – transporter band}} / \text{GFP – transporter RE}.
\]

This allows the relative content of each of the transporters to be compared with each other as well as between different liver samples. All assays were performed using the protein standards detailed in Fig. 1A as well as 30 µg of human liver protein and were performed in triplicate. A calibration curve was performed for each GFP-tagged protein following western blot with transporter-specific antibody to insure that intensity was in the linear range with respect to the applied protein (Fig. 2).

### STATISTICAL ANALYSIS

All data are presented as means ± SD, and statistical significance was set at two-sided \( P < 0.05 \). Significance was evaluated by the Student \( t \) test and Mann-Whitney \( U \) test, as appropriate. Differences between groups were analyzed by using a one-way analysis of variance followed by Bonferroni multiple comparison correction. The statistical significance of correlated data was assessed using the Pearson correlation coefficient and the Spearman correlation coefficient.

### Results

**PATIENT CHARACTERISTICS**

Liver samples were obtained from 21 individuals with cirrhosis and 17 control individuals. Cirrhosis was due to infection with hepatitis C virus in 17 and excess alcohol consumption in 4. Comparative patient characteristics are presented in Table 1.
RELATIVE CONCENTRATIONS OF TRANSPORTERS WITHIN AND AMONG INDIVIDUALS

All transporter assays were performed in triplicate, and deviations from the mean were <10%. The distribution of the five transporters that were studied is shown in Fig. 3 as a heat map showing expression of each transporter in GFP units, as described in Materials and Methods. In this figure, data from control patients and those with cirrhosis are grouped separately and results in patients have been sorted according to their OATP1B1 content, from lowest to highest. Age, sex, and ethnicity of each individual are indicated at the top. These data are also presented as scatter plots for each transporter comparing expression in individual patients with and without cirrhosis (Fig. 4). When all individuals were considered together (Fig. 4A), it was apparent that there is a large spread in expression of each transporter. This spread was seen even when normal and control groups were examined separately (Fig. 4B,C). In control patients, OATP1B1 was the most abundant transporter (Fig. 4B). In the group of patients with cirrhosis, expression of OATP1B1 was reduced and was equivalent to expression of OATP1A2 (Fig. 4C). Expression of both these transporters in patients with cirrhosis was significantly greater than expression of the other transporters that were examined (Fig. 4C). Interestingly, expression of NTCP in the group of patients with cirrhosis was significantly lower than that of the other transporters while it did not differ from expression of OATP1B3 or OATP2B1 in control subjects (Fig. 4B,C). Comparison of transporter expression between controls and patients with cirrhosis is best seen in the side-by-side grouping in Fig. 4D in which significant reductions in expression of OATP1B1 and NTCP are evident. Although there was a trend toward higher expression of OATP1A2 and OATP2B1 in patients with cirrhosis, this was not statistically significant ($P = 0.07, P = 0.12$, respectively). Transporter expression in cirrhotic liver from patients with hepatitis C was similar to that in alcohol-induced cirrhosis (data not shown).

RELATIONSHIP OF SEX, AGE, AND ETHNICITY TO HEPATIC TRANSPORTER EXPRESSION

Although expression of OATP2B1 was significantly ($P < 0.05$) higher in women compared to men in the total (controls plus those with cirrhosis) group

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**FIG. 3.** Heat map showing protein expression in GFP units/30 µg of liver protein for the five transporters in livers from 17 patients without cirrhosis (control) and 21 patients with cirrhosis, as indicated. Results are sorted based on OATP1B1 expression. Age, sex, and ethnicity of each individual are indicated in the color coded region at the top according to the scale on the left. The color gradient indicates the quantitative expression of transporters in GFP units ranging from high expression (red) to low expression (blue), as indicated in the color key at the top of the figure.
of patients, this difference was small and was not seen for the other transporters (Fig. 5A). In control subjects, OATP1A2 expression in women was approximately double that in men \((P < 0.05)\), while there were no significant differences in expression of the other transporters (Fig. 5B). There were no significant differences in transporter expression between men and women in the group of patients with cirrhosis (Fig. 5C). There was no significant correlation of any transporter with age in either controls or subjects with cirrhosis (Fig. 6). Transporter expression was also examined as a function of ethnicity (Fig. 7). The 5 patients whose ethnicity was not provided were excluded from this analysis. When patients with cirrhosis and controls were grouped together (Fig. 7A), OATP1A2 expression in Caucasians was significantly lower than that in Hispanics \((P < 0.05)\). However, there was no significant difference in expression of any transporter in any of the other groupings, although transporter expression in Caucasians tended to be lower than that in African Americans or Hispanics.

**COORDINATE EXPRESSION OF TRANSPORTERS IN INDIVIDUALS**

As seen in the preceding results, there is a great range of transporter expression among individuals. We next examined whether individuals had a random distribution of transporters or whether those individuals with high or low expression of a specific transporter had a similar magnitude of expression of the other transporters. When examined in binary fashion, the level of expression in each patient of one transporter was highly correlated with that of the other transporters, suggesting the possibility of coordinated regulation of protein expression in the liver (Fig. 8).
Discussion

Previous studies indicate that members of the OATP family as well as NTCP are important in hepatic uptake of a variety of endogenous compounds, such as bile acids, steroid hormones, and thyroid hormones, as well as many drugs, including statins, fexofenadine, repaglinide, and methotrexate.\(^8,9,27-29\) In the present study, a method that uses GFP-transporter fusion protein standards was developed to quantify and compare transporter content of human liver. These studies complement previous studies using proteomic technology. The use of tagged transporter standards obviates potential limitations in LC-MS, including variations in proteolytic digestion of proteins\(^{16-19}\) and possible variations in recovery or solubilization of these integral membrane proteins from the membrane preparations that were used\(^{17,18}\).

Methods used in the present study require minimal sample preparation and manipulation and do not depend on quantitative tryptic digestion, membrane fractionation, or protein solubilization, other than in SDS-PAGE sample buffer following homogenization of liver samples. Differences in these parameters may have produced variability in transporter quantification previously\(^{30}\). The use of tagged protein standards in the present study is a complementary procedure that obviates some of these concerns while permitting comparative assessment of transporter expression within a single patient and among patients.

We found that OATP1B1 was the most abundantly expressed transporter. Of interest is the fact that quantitative expression of each transporter spanned almost an order of magnitude when examined across all patients (Fig. 3). This large range of expression indicates that hepatic uptake of specific compounds could be very variable among individuals and difficult to predict in a given individual. This could result in significant differences in hepatic extraction of ligands among individuals, with the potential for systemic toxicity when uptake is reduced or blunted systemic therapeutic response with high uptake. Consistent with this are findings in previous studies showing that reduced expression or activity of OATP1B1 and other transporters results in reduced hepatic uptake of ligand drugs \(\textit{in vivo}\) and \(\textit{in vitro}\).\(^{31,32}\) There have been a number of studies seeking to mathematically model hepatic drug transport based on \(\textit{in vitro}\) quantitation of transporter activity.\(^{33,34}\) However, the substantial heterogeneity of transporter expression as observed in our study indicates the difficulty of predicting drug uptake and drug–drug interactions in individuals using these models. Interestingly, the interindividual variability of transporter expression in this study is substantially larger than that described in reports using mass spectroscopic technology.\(^{17}\)

Whether this is due to differences in the patient populations that were studied or differences in methodology\(^{30}\) is unclear. Studies that were performed using MS are in agreement with the present study that
Fig. 6. Transporter expression in the liver as a function of age. Transporter expression was calculated and expressed in GFP units/30 µg liver protein, as described in Materials and Methods. Results are presented as scatter plots of average transporter expression in determinations performed in triplicate for each individual. Statistical analysis was by the Pearson test ($R$) in scatter plots from total patients (upper panels) and by the Spearman test ($Rs$) in plots of data from controls (middle panels) and subjects with cirrhosis (lower panels). Correlation coefficients, $P$ values, and $n$ are indicated on each panel.
Fig. 7. Transporter expression in the liver as a function of ethnicity. Transporter expression was calculated and expressed in GFP units/30 µg liver protein, as described in Materials and Methods. Results are presented as scatter plots of average transporter expression in determinations performed in triplicate for each individual. Patients self-identified as African American, Hispanic, and Caucasian are indicated. Five patients declined to provide this information, and their data were not included in this analysis. Plots are presented for (A) the group of all 33 subjects (AA, n = 12; H, n = 14; C, n = 7); (B) control subjects without cirrhosis (AA, n = 7; H, n = 6; C, n = 4); (C) subjects with cirrhosis (AA, n = 5; H, n = 8; C, n = 3). Bars represent means ± S.D. Statistical difference was determined by the Mann-Whitney U test; ●, control; ○, cirrhosis. Abbreviations: AA, African American; C, Caucasian; H, Hispanic.
FIG. 8. Binary comparison of hepatic transporter expression. Transporter expression was calculated and expressed in GFP units/30 µg liver protein, as described in Materials and Methods. Results are presented as scatter plots of average transporter expression in determinations performed in triplicate for each individual. Each point represents results in a single individual, and comparison of transporter expression was examined for all possible pairs. Statistical analysis was by the Pearson test. Correlation coefficients ($R$), $P$ values, and $n$ are indicated for each panel. Significant correlation was detected between expression of all transporters with each other, suggesting coordinate regulation.
OATP1B1 was the most abundantly expressed transporter when compared to expression of OATP1B3 and OATP2B1. However, some studies using quantification by MS found that OATP2B1 expression was substantially less than that of OATP1B1, similar to what was found in the present study, while other studies showed that they were little different from each other (reviewed in Prasad et al. [17]). Again, it is possible that this represents variability in expression in the study populations.

It is intriguing in the present study that the variability in quantitative expression of transporters is seen primarily among individuals. Expression of the five transporters in a single subject shows little variability (Fig. 3). That is, some subjects are high expressers while others are low expressers. This can be best seen in the correlation plots (Fig. 8) in which binary comparison of transporter expression shows a very significant correlation of the levels of each transporter with levels of the other four transporters in a given individual. Similar coordinate expression of OATP1B1, OATP1B3, and OATP2B1 with highly variable expression among individuals was also described by Nies et al. [15]. These results suggest that there could be a mechanism for coordinate expression of these transporters. Three of the transporters (OATP1A2, OATP1B1, and OATP1B3) are localized near each other on chromosome 12, suggesting that they could share common regulatory elements. As OATP2B1 and NTCP are localized to two different chromosomes (11 and 14, respectively), the possibility of common regulatory mechanisms related to trans-acting factors that interact with DNA–protein binding domains or posttranscriptional regulation of messenger RNA stability by binding to an element on the RNA molecule should be considered. Although candidate factors have been suggested, further investigation will be required to elucidate the importance of these potential regulatory mechanistic components.

Although we were expecting to observe substantial differences in transporter expression in cirrhotic compared to noncirrhotic liver, this was not the case. Expressions of OATP1B1 and NTCP were significantly reduced in cirrhotic liver. Although these differences were small (Fig. 4D), they could result in reduced uptake of their ligands, adding to effects produced by vascular shunting around the liver from portal hypertension as well as loss of sinusoidal endothelial fenestrations, reducing access of protein-bound ligands to the space of Disse and hepatocyte basolateral plasma membrane transporters. It should also be noted that most individuals from whom tissue was obtained were on various medications that could potentially increase or decrease transporter expression, although such effects have not been well characterized in human liver. Our results also differ from those reported using mass spectroscopic analysis in which there was no difference in OATP1B1 expression in cirrhosis while expression of OATP1B3 was reduced and expression of OATP2B1 was increased; we found that neither was changed. It is also noteworthy that there was little sex difference in transporter expression, although OATP1A2 was significantly elevated in women without cirrhosis compared to men (Fig. 5B). The clinical importance of this finding remains to be established. There were also no statistically detectable differences in transporter expression between African Americans, Hispanics, or Caucasians, with or without cirrhosis, although our sample size was limited and the range of expression within each group was large (Fig. 7).

In the present study, total expression of transporter proteins was determined in 30 µg of liver lysate protein. It must be recognized that this represents only part of the picture when assessing the ability of the liver to take up substrate ligands. Total hepatic transport capacity is a function of liver size and protein content, parameters that are difficult to quantify. In addition, it is possible that a subset of the study subjects may have polymorphisms in genes encoding specific transporters. The protein products resulting from such polymorphisms could potentially have reduced transport activity due to factors such as stereochemical interference with ligand binding or altered trafficking to the plasma membrane from intracellular locations. Reduced trafficking to the cell surface could be due to changes in the glycosylation or phosphorylation states or altered interaction with chaperone proteins, such as PDZK1. These parameters are important for assessment of overall transporter function and, while beyond the scope of the present report, will be the subject of future study.

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