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Original article

The reference liver – ABC and SLC drug transporters in healthy donor and metastatic livers

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ABSTRACT

Background: Analysis of results and conclusions in studies dedicated to pathology of the liver are usually based on comparison of pathological liver specimens and control/reference (considered as healthy) tissues. There are two main sources of the control liver samples used as the reference livers, i.e. deceased organ donor livers and non-tumorous tissue from metastatic livers, which are also applied for drug transporter investigations. However, no information has yet been published on drug transporters in these two major types of reference livers.
Methods: We explored ABC (P-gp, MRP1, MRP2, MRP3, MRP4, BCRP, BSEP) and SLC (NTCP, MCT1, OCT1, OCT3, OAT2, OATP1B1, OATP1B3, OATP2B1) family transporters expression (qPCR) and protein abundance (LC–MS/MS) in healthy donors ($n = 9$) and metastatic ($n = 13$) livers.
Results: The analysis of mRNA content revealed significant differences in *ABCB11, ABCC1, ABCG2, SLC10A1, SLC16A1, SLCO1B1* and *SLCO2B1* gene expression between livers from organ donors and patients who underwent surgical resection of metastatic tumors. The protein abundance of NTCP was significantly higher, whereas of P-gp significantly lower in non-tumorous tissues from metastatic livers. Greater inter-individual variability in protein abundance of all studied transporters in subjects with metastatic colon cancer was also observed.
Conclusions: The results suggest that final conclusions in liver pathology studies may depend on the reference liver tissue used, especially in gene expression studies.

Introduction

Absorption, distribution and bioavailability of many xenobiotics, including drugs, strongly depend on function of transporter proteins in different tissues and organs. Considerable scientific progress towards understanding the role of membrane transporters in drug pharmacokinetics as well as drug-drug interactions has led to the publication of guidelines by regulatory agencies, including the Food and Drug Administration (FDA) and European Medicines Agency (EMA). Those guidelines indicate the importance of inclusion of data on the transporters abundance in drug development process [1]. A substantial progress in protein analysis methods in the last decade, mainly utilizing mass-spectrometry-based assays, enabled absolute and accurate quantitation of drug transporters in tissue specimens, and substantially contributed to the definition of their biological functions [2]. A number of papers have been published, focusing on altered drug transporters content in various liver pathologies, i.e. viral hepatitis, hepatic cancer, alcoholic, immunological and cholestatic liver diseases, non-alcoholic steatohepatitis [3–7]. Those studies provided both mRNA expression information as well as semi-quantitative analysis of proteins (mainly Western blotting), and more recent ones, quantitative protein abundance data. Regardless the method used, final conclusions are usually drawn from comparisons of pathological liver specimens and control (considered as healthy) tissues. There are two main sources of the control liver samples used as the reference livers in published studies, i.e. donor livers [3,4] and non-tumorous tissue from metastatic livers [5,4–7], also applied for drug transporter investigations. Some of the studies included also a control group composed of both, liver tissue

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harvested during tumor resection and several liver biopsy specimens [8,9]. Non-tumorous tissue from cancer patients or liver samples from deceased organ donors constitute an accessible source of liver tissue for proteomic and functional studies, but may not reflect entirely the same functional liver state. Thus, the selection of the reference liver tissue may impact final conclusions in transporter expression/protein abundance, functional studies which provide drug transporter characteristics as well as data used for pharmacokinetic modelling [10–12]. Therefore, the present study aimed, for the first time, to characterize drug transporter expression and protein abundance in the two most frequently used types of reference liver tissues, namely liver tissue from organ donors and non-tumorous tissues from patients with metastatic (colon cancer) livers.

Materials and methods

Liver samples

Tissues was obtained from two groups of individuals: 1) organ donors (n = 9), and 2) patients undergoing surgical resection of liver metastases (n = 13). Deceased organ donors died from intracranial bleeding or head injury, and were free from chronic diseases (except for one atrial fibrillation subject medicated with amiodarone). After brain death had been determined, deceased patients were reported as potential organ donors to national center, and subsequently, transplantation team was sent to the place of patient death for organ collection. A small sample of liver tissue for the current study was taken from biopsy collected for histopathological examination of explanted liver. All subjects received short-term emergency drugs before tissue dissection, including dopamine, dobutamine, epinephrine, lidocaine, sodium nitroprusside, vasopressin, cephalosporines, gentamycin, vancomycin, clindamycin, insulin and mannitol. The samples of non-tumorous liver tissue, located at least 5 cm aside from metastatic tumors, were collected during resection of metastatic colon adenocarcinoma in patients free from chronic diseases (except from one patient with hypertension and prostate hypertrophy medicated with bisoprolol, furosemide, tamsulosin; one patient with hypertension medicated with bepridil, and one patient with hypertension medicated with amlodipine). During the operation the patients were anesthetized with sevoflurane, propofol, rocuronium, fentanyl, oxycodone, midazolam. Characteristics of both study cohorts are given in Table 1. The collected liver tissues didn't show any signs of inflammation or necrosis as confirmed by histological examination. Donor tissue biopsies were taken not later than 30–45 min after blood flow arrest and metastatic liver tissues directly after dissection. Afterwards, the specimens were immediately snap frozen in liquid nitrogen for protein analysis or stored in RNAlater (Applied Biosystems, USA) for RNA analysis. All samples were stored at −80 °C before processing. The study was carried out in accordance with the Declaration of Helsinki and the study protocol was approved by the local Bioethics Committee of the Pomeranian Medical University.

mRNA quantification

Total RNA was isolated from 20 mg of tissue specimen by means of mirVana kit (ThermoFisher Scientific, USA – donors' group) or Direct-zol RNA MiniPrep kit (Zymo Research, USA), followed by on-column DNA digestion step. RNA concentration and purity was subsequently assessed using DS-11 FX spectrophotometer (Denovix, USA). Reverse transcription was performed using SuperScript VILO Master Mix (Thermo Fisher Scientific, USA), using 500 ng for 20 u L of reaction volume, according to a protocol from the supplier. Relative expression of transporter gene expression was determined by means of real-time PCR, using TaqMan Fast Advanced Master Mix and pre-validated TaqMan assays (Thermo Fisher Scientific, USA). Details of TaqMan assays are provided in Supplementary Table 1. All reactions were performed in volume of 10 μL in ViiA 7 Real-Time PCR System (Life Technologies, USA). Threshold values were unified for all the genes, and C_T values were used to calculate relative transcript concentrations (ΔC_T method). Five more stably expressed reference controls (housekeeping genes: *GAPDH, PPIA, HMBS, RPLP0*, and *RPS9*) were selected from 10 candidates initially considered, and mean C_T value was used as a reference for quantification of relative expression of transporter genes. Additionally, *IL1B, IL6, IL10* and *TNF* gene expression was evaluated in all liver samples.

Protein quantification by LC–MS/MS

Tissues placed in liquid nitrogen were mechanically disrupted in a stainless steel mortar system. Approximately 40 mg tissue powder of each sample was lysed with 1 mL of 0.2% SDS and 5 mM EDTA containing 5 μL/mL Protease Inhibitor Cocktail Set III (Merck, Darmstadt, Germany) for 30 min at 4 °C on a platform shaker with 40 rpm (Polymax 1040, Heidolph, Germany). Total protein content of the whole tissue lysates was determined by bicinchoninic acid assay (Thermo Fisher, USA) and 100 μg of each sample was processed using filter aided sample preparation (FASP) based on the previously published protocol [13]. Protein quantification of ABC transporters (P-gp, MRP1, MRP2, MRP3, MRP4, BCRP, BSEP) and SLC transporters (NTCP, MCT1, OCT1, OCT3, OAT2, OATP1B1, OATP1B3, OATP2B1) were measured by mass spectrometry-based targeted proteomics using a validated LC – MS/MS method [14]. LC–MS/MS analyses were conducted on API4000 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) coupled to a Shimadzu LC (SLC-10 A VP) system (Shimadzu, USA) and an HTS PAL LEAP autosampler (LEAP Technologies, USA). After injection of 20 μl sample, the chromatography was performed with a gradient elution using the Kinetex

Table 1
Clinical characteristics of the study groups (mean ± SD, range).

	Organ donors (n = 9)	Patients with metastatic liver (n = 13)	*p*[a]
Age (years)	47.2 ± 12.8 (19-60)	64.1 ± 8.3 (53-77)	*0.001*
Sex (males/females)	6/3	9/4	*0.735*
Total bilirubin [<1.23 mg/dL]	0.79 ± 0.73 (0.1-2.15)	0.55 ± 0.26 (0.20-1.06)	*0.931*
AST [5-40 IU/L]	33 ± 11 (12-219)	37 ± 19 (11-65)	*0.940*
ALT [5-40 IU/L]	23 ± 6 (13-89)	29 ± 18 (10-55)	*0.823*
Serum total protein [6.0-8.0 g/dL]	5.2 ± 1.2 (3.3-6.4)	6.8 ± 0.7 (5.2-7.5)	*0.004*
Serum albumin [3.5-5.5 g/dL]	2.9 ± 0.9 (1.5-3.6)	3.8 ± 0.4 (3.4-4.4)	*0.019*
INR [0.8-1.2]	1.24 ± 0.14 (1.0-1.4)	1.13 ± 0.21 (0.9-1.7)	*0.075*
APTT [26-36 s]	37.4 ± 12.2 (22.0-62.4)	32.6 ± 10.1 (20.8-53.3)	*0.315*
Creatinine [0.6-1.3 mg/dl]	0.99 ± 0.41 (0.6-1.84)	0.90 ± 0.23 (0.54-1.34)	*0.940*

[a] *p* value calculated by means of Mann-Whitney U-test, except for sex ratio (Chi^2 test with Yate's correction); [] – normal range.

C18 column (100 × 3.0 mm, 2.6 μm, Phenomenex, USA) and water with 0.1% FA (solvent A) and acetonitrile with 0.1% FA (solvent B) as mobile phases at a flow rate of 0.5 ml/min. With the exception of MRP1, MRP4, BSEP, MCT1, NTCP, OAT2 and OCT3 (no major drug transporters), all remaining proteins were analyzed by using two proteospecific peptides. In each case, one peptide was used for quantification whereas the other served as qualifier verifying the presence of the specific protein. For all peptides and their isotope-labeled internal standards, three mass transitions were used for quantitative analysis, respectively. The calculated protein values represent the mean of at least 2–3 mass transitions/peptide. All the peptide standards, as well as their respective isotope-labelled internal standard were ordered from SpikeTides, JPT Peptide Technologies (Germany). The details of the procedure, used peptides and mass transitions are given in the Supplementary Table 4.

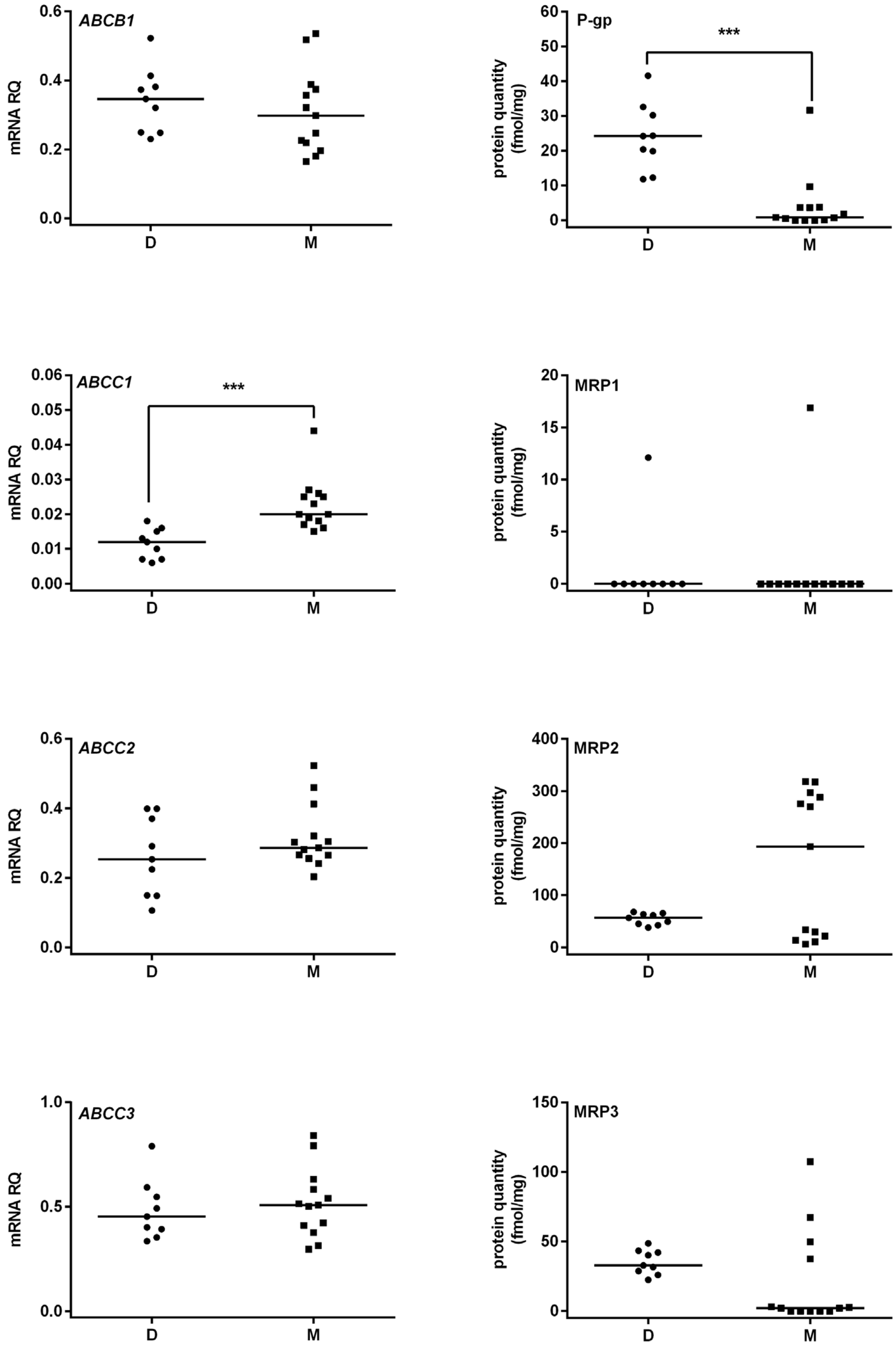

Fig. 1. The mRNA and protein content of ABC transporters (ABCB1, ABCC1, ABCC2 and ABCC3) in livers from organ donors (D) and patients undergoing metastatic tumor resection (M). Horizontal bars represent median values for each group. Values for mRNA are shown as relative quantity in relation to mean expression of five housekeeping genes (*GAPDH, PPIA, HMBS, RPLP0*, and *RPS9)*. Significant *p* values are marked with asterisks ($^{***}p < 0.001$).

Statistical methods

Distribution of the analyzed variables (i.e. mRNA and protein content) was tested for normality using Shapiro-Wilk test. Due to significant deviation from normal distribution, differences between study groups were further evaluated by means of non-parametric Mann-Whitney *U* test. Correlation between mRNA and protein levels was measured with Spearman's rank correlation coefficient. All the calculations were performed using Statistica 13.1 software package (TIBCO Software Inc., Palo Alto, CA, USA).

Results

All of the studied transporter genes were expressed at detectable levels in all the analyzed samples at mRNA level by means of applied rt-PCR method ($C_T<35$). The lowest expression was observed for *ABCC4, ABCC1, ABCG2, SLC22A3*, and *SLC22A18* (less than 10% of mean expression for the house-keeping reference genes), while *SLC22A1* mRNA levels were the highest in both study groups. The analysis of mRNA content revealed significant differences in *ABCB11, ABCC1, ABCG2, SLC10A1, SLC16A1, SLCO1B1* and *SLCO2B1* gene expression between livers from organ donors and patients undergoing surgical resection of metastatic tumors. When median mRNA quantity in liver donors was set as reference (100%), median expression of those genes in metastatic livers was significantly higher (179% for *ABCB11*, 167% for *ABCC1*, 442% for *ABCG2*, 437% for *SLC10A1*, 278% for *SLC16A1*, 224% for *SLCO1B1*, and 172% for *SLCO2B1*). No significant differences were observed in the case of the other studied transporter genes. The results of mRNA quantification are presented in Figs. 1–4 and Supplementary Table 2.

The protein abundance analysis revealed that MRP4 was below the detection limit in all liver samples, while MRP1 and BCRP were quantified only in single individuals. OCT3 levels were also below detection limit in a significant number of samples from both study groups, while P-gp and MRP3 were absent in some livers (>2), but only in metastatic livers. The protein abundance (mean and median) of most of the transporters was higher in livers from cancer patients than in organ donors, but the difference was significant only in the case of NTCP (SLC10A1) – mean value in metastases *vs.* donors group: 114.5 ± 177.8 *vs.* 31.8 ± 9.5 fmol/mg, median value: 88.8 *vs.* 32.5 fmol/mg, $p =$ 0.001, respectively. That observation was in concordance with mRNA analysis results. In contrast, protein abundance of P-gp (ABCB1) was significantly higher in the donors group (mean value in metastatic *vs.* donors group: 4.3 ± 8.3 *vs.* 24.2 ± 9.1 fmol/mg, median value: 0.9 *vs.* 24.2

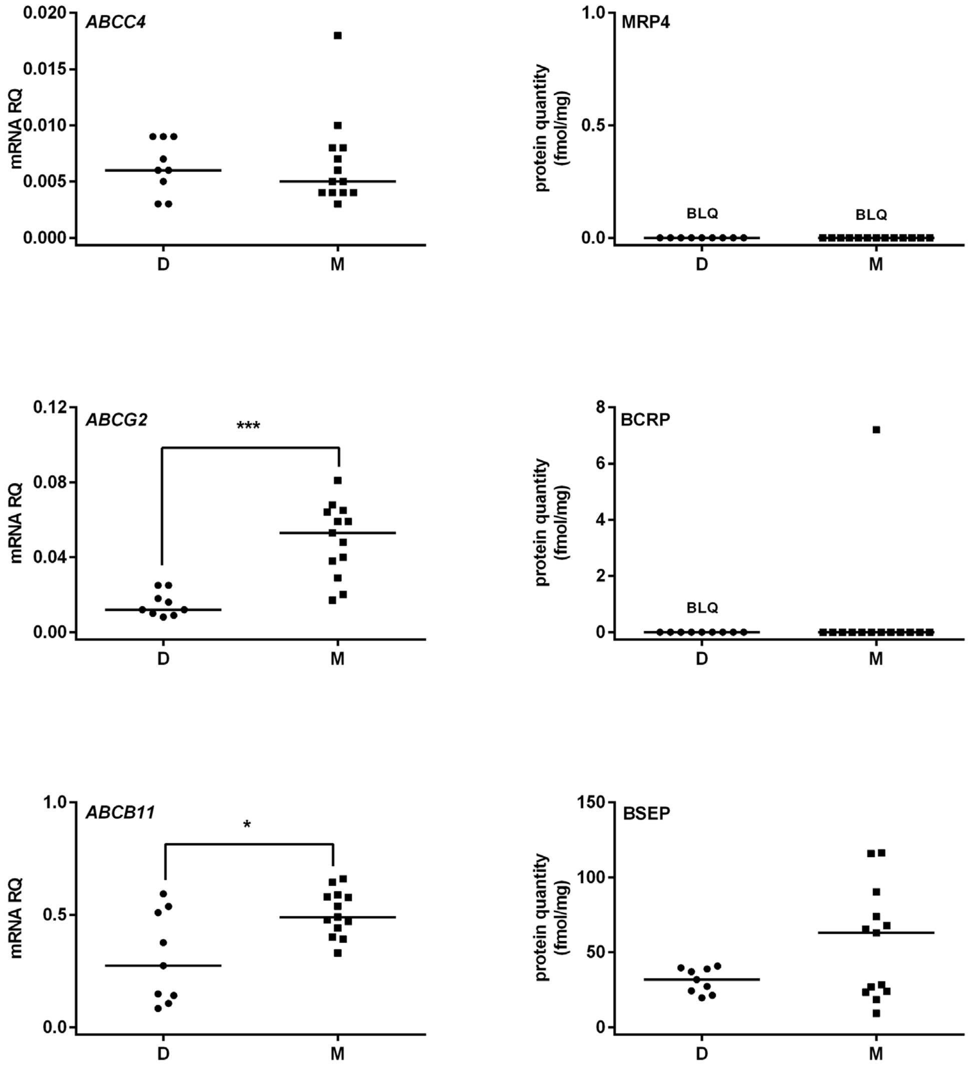

Fig. 2. The mRNA and protein content of ABC transporters (ABCC4, ABCG2 and ABCB11) in livers from organ donors (D) and patients undergoing metastatic tumor resection (M). Horizontal bars represent median values for each group. Values for mRNA are shown as relative quantity in relation to mean expression of five housekeeping genes (*GAPDH, PPIA, HMBS, RPLP0*, and *RPS9*). Significant *p* values are marked with asterisks ($^{*}p < 0.05$, $^{***}p < 0.001$).

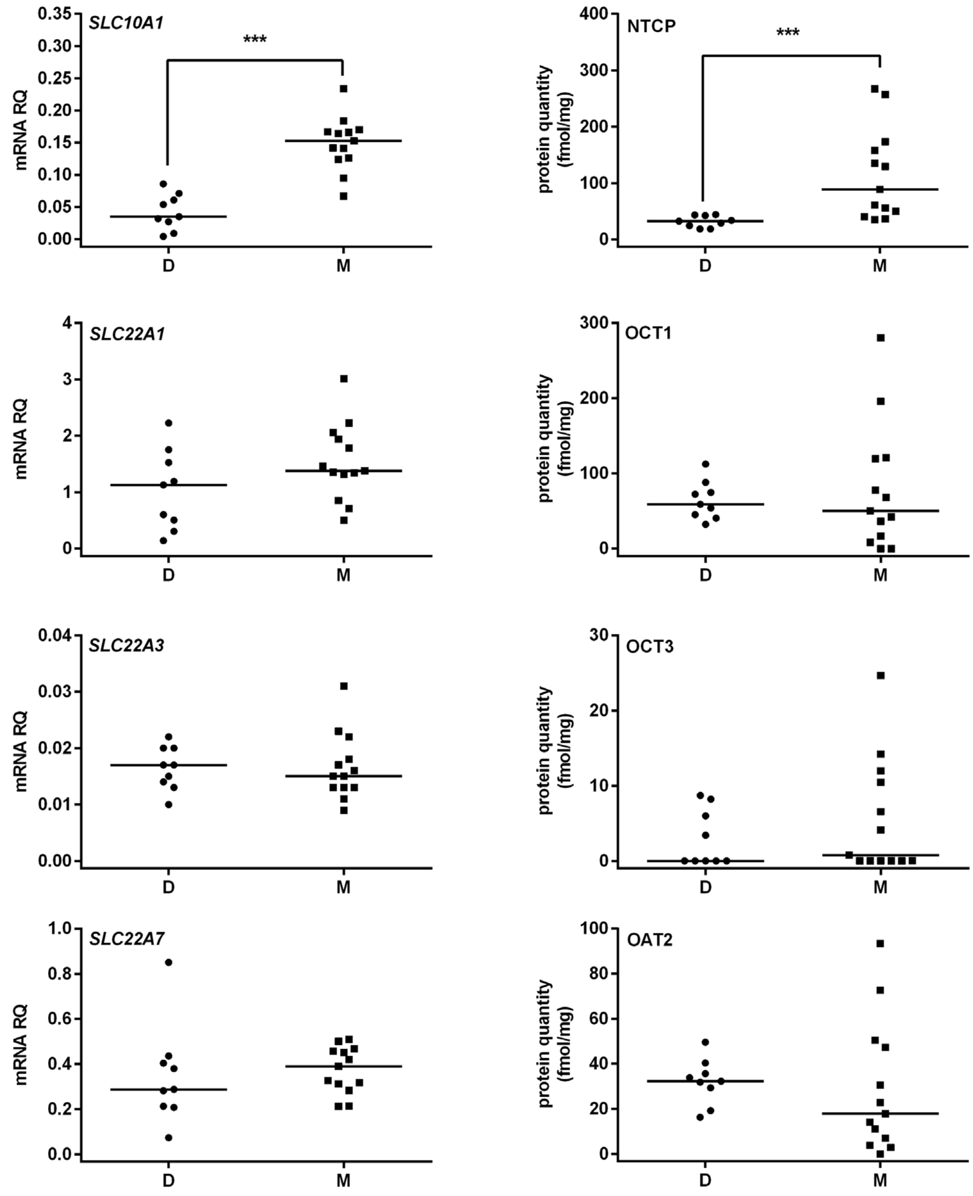

Fig. 3. The mRNA and protein content of SLC transporters (SLC10A1, SLC22A1, SLC22A3, SLC22A7) in livers from organ donors (D) and patients undergoing metastatic tumor resection (M). Horizontal bars represent median values for each group. Values for mRNA are shown as relative quantity in relation to mean expression of five housekeeping genes (*GAPDH, PPIA, HMBS, RPLP0*, and *RPS9*). Significant p values are marked with asterisks (***$p < 0.001$).

fmol/mg, p = 6*10-4), despite similar mRNA expression in both groups. Organ donors were also characterized by numerically higher OATP1B3 (SLCO1B3) protein content, but the difference was not significant (mean value in metastatic *vs.* donors livers: 60.0 ± 53.1 *vs.* 116.3 ± 60.8 fmol/mg, median value: 56.0 *vs.* 114.1 fmol/mg, p = 0.095). The results of protein analysis are presented in Figs. 1–4 and Supplementary Table 3.

Interindividual variability in protein abundance was greater in metastatic livers for all analyzed transporters, as documented by a higher covariance coefficient (Supplementary Table 3). Additionally, there were significant ($0.5 < r < 0.8$) correlations between mRNA expression and protein abundance for *ABCB11*/BSEP, *SLC22A7*/OAT2 and *SLCO1B13*/OATP1B3 (negative) in donor livers, and *SLC22A1*/OCT1, *SLC22A3*/OCT3, *SLC22A7*/ OAT2 and *SLCO1B3*/ OATP1B3 observed in metastatic livers (Table 2). Protein levels of the plasma membrane marker Na+/K + ATPase levels were similar in both groups (results not shown).

Cytokine gene expression was evaluated in liver samples from both groups. Following mean relative mRNA levels of evaluated genes were observed in metastatic liver patients, while compared to mean value in organ donors: 145% for I*L1B*, 70% for *IL6,* 262% for *IL10*, and 242% for *TNF*. The differences between groups were only significant in case of *TNF* (p = 0.021, Supplementary Fig. 1).

Discussion

In the current study, we compared for the first time drug transporter expression and protein abundance in two sets of liver samples, i.e. non-tumoral liver tissue from patients undergoing resection of colon cancer metastases and liver samples from organ donors (all were also liver donors), which are widely used as the reference, presumably healthy, livers [3–6]. Moreover, they are also utilized to estimate transporter expression/abundance in general population [10–12]. The present study results show several

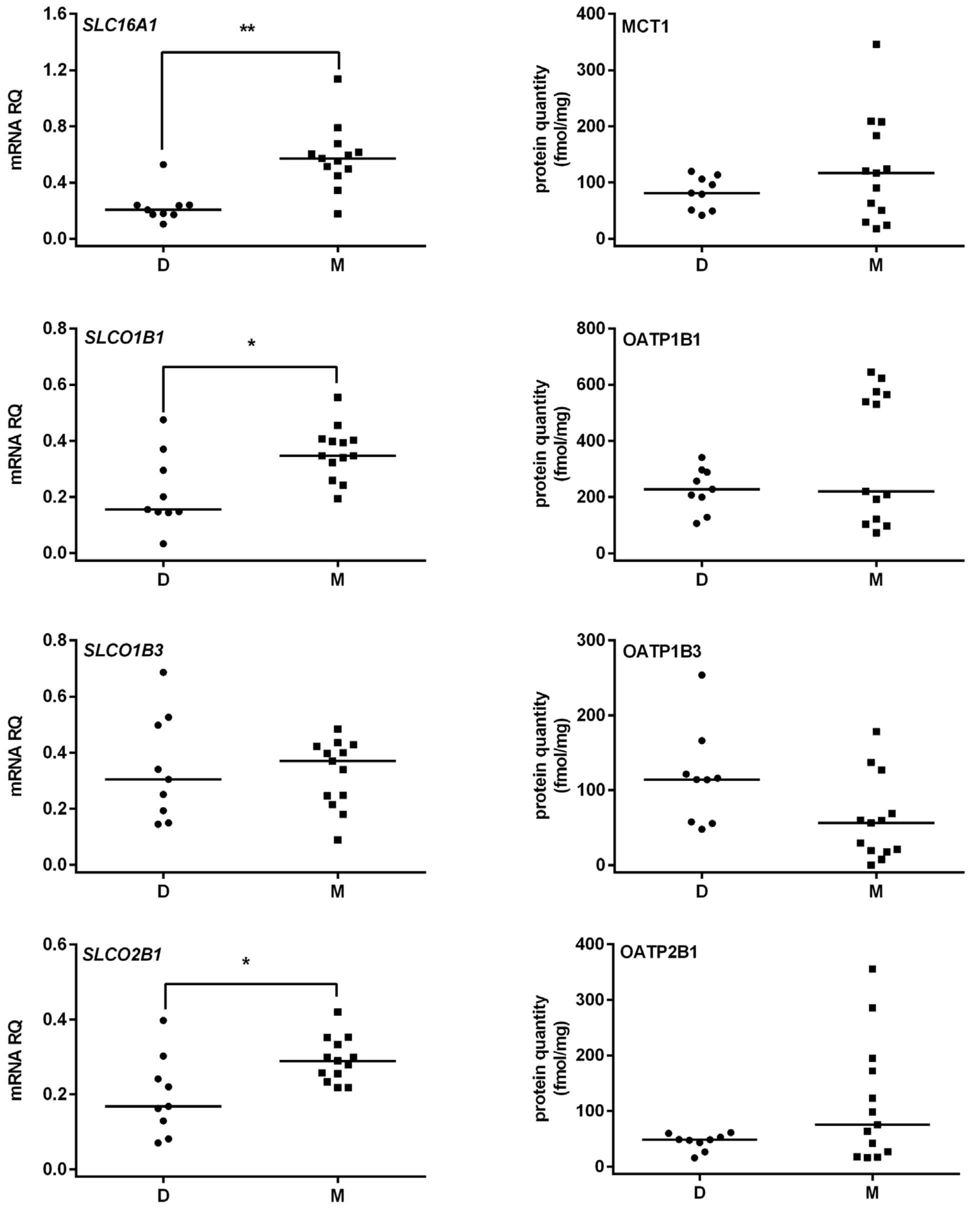

Fig. 4. The mRNA and protein content of SLC transporters (SLC16A1, SLCO1B1, SLCO1B3 and SLCO2B1) in livers from organ donors (D) and patients undergoing metastatic tumor resection (M). Horizontal bars represent median values for each group. Values for mRNA are shown as relative quantity in relation to mean expression of five housekeeping genes (*GAPDH, PPIA, HMBS, RPLP0*, and *RPS9*). Significant *p* values are marked with asterisks (*$p < 0.05$; **$p < 0.01$).

significant differences between the aforementioned types of liver tissues, which may potentially impact conclusions, depending on the reference tissue used.

There were significant differences in mRNA expression levels observed for 7 out of 15 analyzed genes. This observation suggests, that selection of a reference/control group may influence interpretation of gene expression studies performed in liver pathologies. This study directly evidenced that analysis of *ABCB11, ABCC1, ABCG2, SLC10A1, SLC16A1, SLCO1B1* and *SLCO2B1* expression studies may be affected by the type of the reference liver used.

As for protein abundance, with structure being more stable than mRNA structures, significant differences were noted for only two studied transporters, namely P-gp (MDR1, ABCB1), one of the most important drug transporters (higher values in donors) as well as NTCP (SLC10A1), a sodium-dependent uptake transporter expressed in the basolateral membrane of human hepatocytes, primarily responsible for the uptake of bile acids from the sinusoids (higher values in metastatic liver tissue) [15,16]. Combining the changes in mRNA expression and protein abundance data of *SLC10A1*/NTCP and *ABCB1*/P-gp it can be observed that mRNA levels paralleled protein information for NTCP in both types of the studied livers (significantly lower levels in donor livers), whereas levels of mRNA were comparable in donor and metastatic liver tissues, but the protein abundance was significantly different (higher in donor livers). The latter observation suggests that analysis of *ABCB1* mRNA expression results and P-gp protein (especially when quantitative methods are used) may lead to different conclusions depending on whether donor livers or metastatic livers are taken as reference tissues.

As the consequence of different abundance of the studied drug transporters in donor and nontumoral metastatic liver tissues, the relative transporter contribution and the rank order was altered. In donor livers the transporters could be ranked: OATP1B1 (30.5%)> OATP1B3 (15.5%)> MCT1 (10.9%)> OCT1 (8.6%)> MRP2 (7.3%)> OATP2B1 (6.0%)> MRP3 (4.7%)> OAT2 (4.3%)> NTCP = BSEP (4.2%)> P-gp (3.2%)> OCT3 (0.4%), whereas in metastatic livers as follows:

Table 2
Correlation between RNA and protein quantity of ABC and SLC transporters in human liver samples.

mRNA *vs.* protein	M n = 13	D n = 9
P-gp	0.492	0.250
BSEP	−0.357	-0.767^{*}
MRP1	0.000	0.274
MRP2	0.495	0.400
MRP3	0.350	−0.533
MRP4	-[a]	-[a]
BCRP	0.154	-[a]
NTCP	0.467	0.033
MCT1	0.016	0.267
OCT1	0.743^{**}	0.233
OCT3	0.622^{*}	0.511
OAT2	0.654^{*}	0.717^{*}
OATP1B1	0.412	0.117
OATP1B3	0.764^{**}	0.767^{*}
OATP2B1	0.440	0.383

M – non-tumorous liver samples from patients with metastatic colon cancer; D – liver samples from organ donors; Spearman coefficient values are given; [a] protein below quantification; $^{*}p < 0.05$; $^{**}p < 0.01$.

OATP1B1 (23.52%)> MRP2 (10.9%)> MCT1 (8.3%)> OATP2B1 = NTCP (7.8%)> OCT1 (5.3%)> OATP1B3 (4.1%)> BSEP (3.8%)> OCT3 (2.0%)> MRP3 (1.4%)> OCT3(0.4%)> P-gp (0.3%). The most prominent differences were seen in the case of P-gp, which is an important drug transporter, and thus the reference liver values might be of importance in the data analysis.

There are several factors that may be responsible for the observed findings. Organ donors are usually administered emergency drugs, including cardiovascular agents (dobutamine, dopamine, ephedrine, lidocaine, nitroprusside, procainamide, phenylephrine, vasopressin), antibiotics (cephalosporins, clindamycin, ampicillin/sulbactam, gentamicin, vancomycin), insulin and mannitol. In the case of subjects with brain injury, they can additionally receive dexamethasone and phenytoin, drugs with proven influence on drug metabolizing enzyme and transporter gene expression (influence depends on duration of drug treatment) [17,18]. Some donors, especially those deceased in accidents, experience multiorgan trauma (bone fractures and muscle injuries), which may result in release of not yet defined factors affecting transporter gene expression (more prominent changes at gene expression levels were observed in the present studies).

Additionally, donor liver samples used in several reports might present some abnormalities, like hepatic steatosis or fibrotic changes (this is not the case of the current study), which could further influence the reported conclusions [10,11,19].

Metastatic patients are administered general anesthesia drugs (in the present study: sevoflurane, propofol, rocuronium, fentanyl), and in the current study colon cancer patients were significantly older than organ donors. However, there is no direct data available on age- related NCTP and P-gp abundance changes in the analyzed samples. The P-gp quantification through the age interval from 7 to 70 years revealed that age and P-gp protein expression were not correlated [11]. Based on the latter observation it might be suggested that age should not be a factor determining the observed differences in P-gp levels in the donor and nontumoral metastatic livers.

As for liver samples obtained from patients undergoing metastatic colon cancer resection, malignancy might be considered as a factor with potential systemic effects, including hepatic transporter expression. The decrease in *ABCB1* gene expression in normal intestinal tissue in patients with colorectal cancer (when compared with healthy individuals) was also documented [20]. Evidence exists that serum profile of circulating cytokines is significantly altered in colorectal cancer [21] and it may probably affect functions of peripheral tissues. Some inflammatory mediators (e.g. interleukin-6, IL-6) were demonstrated to suppress *ABCB1* expression in primary hepatocytes, as well as in HepaRG cell line, and significant elevation of IL-6 colon cancer patients was reported [21–23]. We also documented significant reduction of *ABCB1* transcription in another general inflammatory state, i.e. in mucosal biopsy specimens from patients with ulcerative colitis, and significant reduction of P-gp protein levels in Caco-2 cells under IFN-γ exposure [24]. Hence, reduced abundance of P-gp in normal liver tissue, observed in the current study may potentially result from generalized cancerous process and systemic changes in immune system. Unfortunately, plasma samples from patients involved in the current study were not available, which may be considered as one of the study limitations. Analysis of *IL1B*, *IL6*, *IL10* and *TNF* relative expression was performed in the same liver samples that had been investigated for drug transporters gene expression. One should be aware of fact that measurement of *in situ* cytokine gene expression does not necessary reflect systemic changes in protein levels. However, we observed significantly increased expression of *TNF* in liver tissue from metastatic patients (242% of mean value for organ donor group). Since TNF is mainly produced by immune cells, acting as a potent mediator of inflammation, its altered expression observed in the current study may be related to immunological processes, as consequence of metastatic liver disease (due to colon cancer).

We also observed a significantly higher *SLC10A1* gene expression and NTCP protein level among metastatic individuals *vs.* donor livers. Na+/taurocholate co-transporting polypeptide (NTCP), which is expressed on the basolateral membrane of hepatocytes and is primarily responsible for the uptake of bile acids from the sinusoids, has been recently recognized as the main HBV entry receptor [25]. Interestingly, it was demonstrated that SLC10A1 expression and function was suppressed by IL-6 in HepaRG cells and primary hepatocytes [23,26]. However, other factors are known to be involved in NTCP transcriptional regulation, i.e. bile salts, sex hormones and glucocorticoids [27]. Hence, other clinical factors, beyond the presence of tumor, might also be responsible for the observed differences between the analyzed subsets of liver tissues.

The present study also demonstrates that the correlation between gene expression and protein abundance of the studied transporters was different, and the values of correlation coefficient were all below 0.8, for *ABCB11*/BSEP, *SLC22A1*/OCT1 and *SLC22A3*/OCT3 between metastatic and donor livers. These observations, and lack of correlation between mRNA and protein content for most of the analyzed drug transporters suggest that measurement of gene expression at mRNA level may not be a proper tool to determine changes in transporter function in human liver. The findings also suggest that hepatic transporter processing is regulated by other mechanisms beyond transcription. Involvement of microRNA was evidenced for P-gp abundance regulation, which was significantly different in the studied donor and nontumoral metastatic livers [28,29].

Apart from the quantitative differences in transporter levels observed between the donor and metastatic livers, greater interindividual variability in all studied transporters' protein abundance in subjects with metastatic colon cancer was observed, and it was evidenced by a higher covariance coefficient in the case of all the analyzed targets (Figs. 1–4 and Supplementary Table 3). As a consequence, using donor livers as a reference would probably make a study more sensitive and enable detection of even minor differences in case-control studies.

The present study provides for the first time information on drug transporter levels in two different types of liver tissues used as reference livers, i.e. donor and nontumoral metastatic livers. However, some limitations of the study should also be taken into consideration, such as limited number of cases, not fully matched age and medications in both study groups.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.pharep.2019.04.001.

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