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Clinical implications of altered drug transporter abundance/function and PBPK modeling in specific populations: An ITC perspective

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Abbreviations:

CYP: Cytochrome P450; OATP: Organic Anion Transporting Polypeptide; BCRP: Breast Cancer Resistance Protein; P-gp: P-glycoprotein; CP-I: Coproporphyrin I; CP-III: Coproporphyrin III; RI: Renal Impairment; GFR: Glomerular Filtration Rate; CKD: Chronic Kidney Disease; OAT: Organic Anion Transporter; OATP1B: Organic Anion Transporting Polypeptide 1B; MCT: Monocarboxylate Transporter; MRP2: Multidrug Resistance Protein (Type 2); NTCP: Sodium (Na) Taurocholate Cotransporting Polypeptide; OCT1: Organic Cation Transporter (Type 1); NAFLD: Non-Alcoholic Fatty Liver Disease; NASH: Non-Alcoholic Steohepatitis; DAA: Direct Acting Antiviral; HCV: Hepatitis C Virus
HIV: Human Immunodeficiency Virus; AIDS: Acquired Immunodeficiency Syndrome; IVIVE: In Vitro - In Vivo Extrapolation; HBV: Hepatitis B Virus; UGT: Uridine Diphosphate (UDP) Glucuronosyltransferase; BSEP: Bile Salt Efflux Pump; SLCO1B1: Solute Carrier Organic Anion Transporter (Family Member 1B1); PEPT1: Peptide Transporter 1; MATE1/2K: Multi-drug and Toxin Extrusion Protein (Type 1/2K); IND: Investigational New Drug; NDA: New Drug Application; BLA: Biologics License Application; HI: Hepatic impairment; RI: Renal impairment; SEV: Small Extracellular Vesicles; PD: Pharmacodynamic; MDR1: Multidrug Resistance Protein (Type 1); PTCPGK: Proximal Tubular Cells Per Gram of Kidney; CRLM: Colorectal Liver Metastases; CSF: Cerebrospinal Fluid

ABSTRACT

The role of membrane transporters on pharmacokinetics (PK), drug-drug interactions (DDIs), pharmacodynamics (PD), and toxicity of drugs has been broadly recognized. However, our knowledge of modulation of transporter expression and/or function in diseased patient population or specific populations such as pediatrics or pregnancy is still emerging. This white paper highlights recent advances in studying the changes in transporter expression and activity in various diseases (i.e., renal and hepatic impairment, cancer) and some specific populations (i.e., pediatrics and pregnancy) with the focus on clinical implications. Proposed alterations in transporter abundance and/or activity in diseased and specific populations are based on 1) quantitative transporter proteomic data and relative abundance in specific populations *versus* healthy adults, 2) clinical PK, and emerging transporter biomarker and/or pharmacogenomic data, and 3) physiologically based pharmacokinetic (PBPK) modeling and simulation. The potential for altered PK, PD and toxicity in these populations needs to be considered for drugs and their active metabolites in which transporter-mediated uptake/efflux is a major contributor to their absorption, distribution, and elimination pathways and/or associated DDI risk. In addition to best practices, this white paper discusses current challenges and knowledge gaps to study and quantitatively predict the effects of modulation in transporter activity in these populations, together with the perspectives from the International Transporter Consortium (ITC) on future directions.

INTRODUCTION

The role of drug transporters in determining systemic and tissue exposure of drugs and their clinical implications has been broadly recognized.[1-3] However, the knowledge of potential modulation of transporter expression and function in disease and in specific populations and their impact on pharmacokinetics (PK), drug-drug interactions (DDIs), pharmacodynamics (PD), and toxicity of drugs is still emerging. In 2018, the International Transporter Consortium (ITC) published a whitepaper[4], which provided a comprehensive review of changes in transporter expression and activity associated with acute and chronic diseases and discussed regulatory pathways and mechanisms that may affect transporter expression and function. The pathophysiological and biological changes in various disease states and developmental changes in pediatric patients may alter systemic and/or tissue drug exposure (i.e., PK), and subsequently lead to changes in efficacy and toxicity of drugs. Therefore, it is critically important in drug development to understand the changes in drug exposure in these populations from a drug safety and efficacy perspective, as recognized also in draft guidance documents by the U.S. Food and Drug Administration (FDA) suggesting the inclusion of renally impaired subjects in late-stage clinical trials to enhance the diversity of clinical trial populations.[5,6]

Rapid progress has been made over the last few years towards understanding the effects of disease and developmental changes on transporter expression and/or activity in clinical settings at a quantitative level. For example, the ontogeny of clinically relevant drug transporters in pediatric patients and altered transporter activity in other specific populations or different disease states have been reported.[7-10] The application of liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based quantitative proteomics has expanded from quantifying transporter abundance in the tissues of healthy adults to that in diseased and specific populations (e.g., liver diseases and pediatric patients). The data describing relative abundance of major transporter proteins in some of these populations *versus* healthy adults are becoming more available and offer valuable information as quantitative readouts of the changes in transporter expression. In recent years, significant progress has also been made in the identification and validation of endogenous substrates of key drug transporters, such as coproporphyrin I (CP-I) for organic anion transporting polypeptides OATP1B or 4-pyridoxic acid for renal organic anion transporter 1/3 (OAT1/3), to de-risk transporter-mediated DDIs.[11-14] These biomarkers have also demonstrated the potential to serve as useful *in vivo* probes to study the change of transporter function in disease states.[15]

Physiologically-based pharmacokinetic (PBPK) modeling has been considered as a powerful tool to incorporate the multitude of disease and developmental changes (system parameters), in addition to drug-related parameters to predict the combined effects of disease, ontogeny and other covariates on the exposure of drugs.[3,16,17] PBPK predictions can support decisions on the need, timing, and design of clinical studies[3,18,19], and supplement limited clinical data in certain patient subsets when recruitment is incomplete, such as pediatric studies. PBPK modeling can also support the inclusion of the patients in late-phase clinical trials to inform on the need for dose adjustments.[5,6] Applications of PBPK models in diseased and specific populations is a fast-growing area[16,20,21] and has demonstrated

promising predictive performance in certain patient populations. In many instances, existing examples are for drugs predominantly cleared by metabolism, as evidenced by recent PBPK modeling in renally impaired (RI) patients[22] or in pediatric patients with spinal muscular atrophy.[16] In addition, the success of *in vitro-in vivo* extrapolation (IVIVE) of transporter-related parameters and confidence in prospective prediction of transporter-mediated drug disposition and DDIs is still challenging.[3,23] Quality and availability of *in vitro* kinetics and transporter abundance data, as well as complexities in determining the rate-determining step in transporter-mediated clearance pathways contribute to these challenges. Despite these gaps, PBPK models validated with clinical data in healthy subjects (using top-down and middle-out approaches) provide a valuable translation tool to be extended to diseased/specific populations when modulation of system-dependent parameters (e.g., transporter expression/activity) is well defined in the population of interest.

The goals of this white paper are to capture recent advances in the field since publication by Evers et al.[4], with a focus on the clinical implications of the changes in transporter expression and activity for various diseases (e.g., RI, liver diseases, and cancer), and extend it to specific populations (e.g., pediatrics and pregnancy) where recent findings on the changes in transporter expression/ function in these populations are available. Highlighted alterations in transporter abundance and/or activity are based on 1) quantitative transporter proteomic data and relative abundance in specific populations *versus* healthy adults, 2) clinical PK, emerging transporter biomarker and/or pharmacogenomic data, and 3) PBPK modeling and simulation case examples in these populations. Integration of these approaches and best practices is illustrated in sections below for clinically relevant transporters (e.g., modulation of OATs in RI or OATP1B/1B3 and MRP2/MRP3 in non-alcoholic steatohepatitis (NASH) among others). We also discuss knowledge gaps and critical challenges, provide the ITC perspective, and propose future directions.

MODULATION IN TRANSPORTER EXPRESSION AND FUNCTION IN VARIOUS DISEASE STATES

Organ impairment

Renal impairment

Renal impairment is associated with decreased glomerular filtration rate (GFR) and albuminuria, as well as the accumulation of uremic toxins due to the loss of renal function. Chronic kidney disease (CKD), which is characterized as progressive RI, can affect the PK of drugs cleared not only via renal, but also nonrenal pathways[24,25] and requires dose adjustment in some cases (e.g., ganciclovir[26]). The effects of RI on the PK of drugs are complex and associated with multiple biological and pathophysiological changes, such as, increased α1-acid glycoprotein, decreased plasma albumin and hematocrit, and potential changes in gastric emptying time among others.[22] Additionally, modulation of the functional activity of various drug metabolizing enzymes (DME) and transporters has been reported that may impact the PK of respective substrates. While the mechanisms are still poorly understood, accumulated uremic toxins in CKD patients have been proposed to directly or indirectly

affect the expression and function of these transporters (references in the Supplementary Reading List). However, direct evidence of changes in enzyme and transporter expression in RI is currently not available due to the lack of quantitative protein expression data in these populations.

The impact of RI on renal transporters in humans is still not well understood and is often implied from the *ad hoc* analysis of clinical data.[27] A decrease in the overall renal clearance of up to 80-90% has been reported in severe RI and attributed to decreased glomerular filtration and active secretion, particularly for renal OAT substrates[28-30]. Considering multiple renal elimination mechanisms (glomerular filtration, passive diffusion and transporter-mediated active secretion), the effect of RI on the function of renal transporters may be convoluted by reduced GFR in RI patients. Recent PK analysis of 33 drugs that are eliminated substantially by tubular secretion and are substrates of OAT1 or OAT3, suggested that the decrease in unbound tubular secretion clearance exceeds the decline in GFR in severe CKD patients[29,31], in contrast to less pronounced change in OCT2.[30,32] Although direct evidence of changes in renal transporter expression/activity in CKD patients is still lacking, reduced protein expression and activity of several renal uptake transporters was reported in a rat model for chronic kidney failure.[33] These mechanisms have increasingly been explored in PBPK models for RI populations[26,29,34] in order to recover the observed changes in plasma PK/urinary excretion of transporter substrates in RI patients (further details in the PBPK modeling section).

A systematic analysis of clinical PK data in RI patients for a range of drugs that are substrates of various cytochrome P450 (CYP) enzymes and hepatic uptake transporters OATP1B has been reported.[24,25] The analysis of 12 OATP1B substrates indicated a consistent decrease in unbound OATP1B-mediated clearance with increasing RI severity, suggesting a decline in OATP1B activity in CKD patients. However, such analysis may be restricted by the complex ADME mechanisms of drugs investigated, reliance on predicted fraction unbound in plasma ($f_{u,p}$) in patients when measured data are not available, and higher PK variability of certain drugs. Since the publication of the ITC white paper in 2018[4], progress has been made to further elucidate the impact of RI on transporter functional activity in humans by conducting PK studies with various transporter probe drugs (**Table 1**) and endogenous biomarkers (**Table 2**). For example, a microdose cocktail study was conducted in healthy volunteers and patients with mild, moderate, or severe RI, or end-stage renal disease (ESRD) to study the effect of RI on the PK of OATP1B, P-glycoprotein (P-gp), breast cancer resistance protein (BCRP) and CYP3A probe substrates.[15] Increased unbound plasma AUC and $C_{max,u}$ (up to ~2-fold) of pitavastatin (OATP1B), rosuvastatin (OATP1B, BCRP), and atorvastatin (OATP1B, BCRP/P-gp, CYP3A) were observed in severe RI patients (**Table 1**). Change in pitavastatin PK at microdose in RI patients was consistent with that reported at therapeutic dose (**Table 1**), despite the lack of clear trends with increasing RI severity. Plasma baseline exposure of the sensitive and specific OATP1B biomarker CP-I was also increased (**Table 2**), while baseline levels of other OATP1B biomarkers were variable and/or not impacted by RI.[15] A weak, but significant increase in baseline plasma CP-I was also reported in two independent studies in patients with more severe stage 3-5 of CKD[35], as well as in patients with ESRD before kidney transplantation[36] (**Table 2**). A significant negative correlation

was observed between estimated GFR and plasma CP-I concentration[36], further suggesting the association between OATP1B activity and severity of CKD. However, other mechanisms such as decrease in CP-I renal elimination are likely to contribute to increased CP-I baseline in severe CKD.[15]

A decrease in the intestinal P-gp activity was noted in CKD based on a microdose study and dabigatran etexilate (dabigatran prodrug) as intestinal P-gp probe drug.[37] A progressive increase in unbound plasma AUC and $C_{max,u}$ of dabigatran with increased RI severity was attributed to reduced renal clearance of dabigatran by GFR and reduced intestinal P-gp activity.[15] Interestingly, a progressive decrease in the magnitude of DDIs between dabigatran and rifampin, an inhibitor of intestinal P-gp, was also observed with increasing RI severity, suggesting a decreased intestinal P-gp activity of up to 40% in severe RI patients.[15] This observation from a microdose study may not be translatable to the therapeutic dose due to potential saturation of intestinal P-gp at the therapeutic dose.[37,38] Nevertheless, the magnitude of the increase in plasma dabigatran AUC in CKD patients is similar as at the therapeutic dose of dabigatran etexilate (**Table 1**). Thus, microdose data likely represent a maximal decrease in intestinal P-gp activity, and considerations of this disease-related modulation of intestinal P-gp may improve the underprediction of dabigatran PK in severe RI patients using PBPK modeling.[39] The change in the intestinal BCRP in RI patients is inconclusive due to high PK variability of rosuvastatin and complex roles of other transporters in its absorption and elimination (e.g., intestinal OATP2B1, hepatic OATP1B/ BCRP)[15]. It is noteworthy that patients with kidney dysfunction often show impaired gastric myoelectrical activity and delayed gastric emptying (references in Supplementary Reading List). Further work is needed to understand the plausible changes in oral absorption processes with increasing severity of RI to further quantify transporter activity modulation in renal impairment.

Hepatic impairment

Liver cirrhosis is a disease characterized by a progressive decline of liver function associated with morphological and physiological changes. The severity of hepatic impairment (HI) is generally classified according to the Child-Pugh (CP) classification system, which assigns point values according to clinical and biochemical markers of liver function (CP-A, mild; CP-B, moderate; and CP-C, severe).[40] It can also be classified based on the liver pathologies (e.g., disease types, see the section below). In addition to a decline in hepatic blood flow, hematocrit, α1-acid glycoprotein, albumin, functional hepatocytes, and biliary excretion, changes in the expression and activity of DME and transporters in liver and other tissues of HI patients have been reported.[22] It is important to appreciate that HI can be caused by multiple chronic diseases. Recent clinical examples of the effects of various types of liver diseases on PK of several transporter substrates are summarized in **Table 1**. Changes in PK in HI patients for 9 drugs approved after 2017 and identified as substrates of one or multiple transporters are summarized in **Table S1**. Quantitative transporter proteomic data reported in human tissues in patients with numerous liver diseases have been summarized in **Table** 3. Changes in hepatic transporter protein expression based on CP-scores in different liver pathologies have also

been reported.[41] Liver function deterioration (CP-C) resulted in a significant increase in the protein expression of hepatic P-gp and MRP4, but a decrease in MRP2, NTCP, OCT1, OATP1B1, and OATP2B1 relative to healthy liver.[41] Knowledge of the disease-related alterations of transporter protein expression relative to healthy subjects is critical for prospective prediction of the effects of liver diseases on PK and safety/efficacy of drugs when incorporated into PBPK models (as system-dependent parameters). However, this approach assumes that the protein abundance (and corresponding changes) correlate with transporter activity,[42,43] which still needs to be confirmed for multiple transporters. Moreover, the protein expression data are either quantified in crude membrane fractions or in tissue homogenate, which does not consider possible disease-mediated alterations in transporter trafficking to the plasma membrane. Details on the modulation of transporter expression and function and their impact on PK by several liver diseases will be discussed in the section below.

Non-Alcoholic Fatty Liver Disease (NAFLD)

NAFLD is a progressive liver disorder with an estimated global prevalence of about 25%,[44] which ranges from relatively benign steatosis (fat accumulation in the liver) to non-alcoholic steatohepatitis (NASH) – a condition characterized by steatosis in combination with inflammation, hepatocellular damage and fibrosis.[45] Further details of the changes associated with NAFLD, including differences in expression of hepatic metabolic enzymes and microsomal scaling factors, and possible implications on drug disposition and efficacy/toxicity have been summarized recently[46]. Marked changes in the liver function in NASH led to the interest in quantifying hepatic expression and activity of drug transporters and metabolizing enzymes. Early global gene expression analysis revealed a general down-regulation of hepatic uptake transporters in livers from NASH patients.[47] In contrast, hepatic efflux transporters such as MRP3 or MRP4 showed a trend of increasing expression at both the mRNA and protein level with progression of NAFLD.[48,49] Recent LC-MS/MS targeted proteomic studies reported a significantly decreased protein abundance of OATP1B1, OATP1B3, OAT2B1, NTCP and OAT2 in membrane preparations of NASH liver tissues compared to healthy liver (**Table 3**). In contrast, expression of MRP3, a sinusoidal efflux pump, was consistently elevated in NASH liver samples using immunoblot and targeted proteomics.[21,50] In addition to the noted differential modulation in transporter expression, other complex events such as the downregulation of N-linked glycosylation of proteins with an accompanying increase in non-glycosylated protein levels have been suggested for several drug transporters in NASH[51]. Immunoblot analysis of liver tissues revealed a significant loss of glycosylation of OATPs, NTCP, and MRP2 in NASH[51]; such impairment in glycosylation may contribute to decreased plasma membrane localization and thus the function of drug transporters (see Supplementary Reading List for additional information).

Clinical PK data in patients with steatosis/NASH are limited (**Table 1**)[4]. Nonetheless, when available, findings from expression data in tissues samples are generally aligned with clinical observations describing PK changes in steatosis/NASH. For example, ^{99m}Tc-mebrofenin plasma and liver exposure increased in patients with biopsy-confirmed NASH indicating a functional decrease in OATP1B-mediated uptake and MRP2-mediated biliary excretion.[52] Decreased hepatic uptake via

OATP1B1/1B3 and NTCP and biliary excretion and/or sinusoidal efflux via MRP2/MRP3 is a likely mechanism for elevated plasma concentrations of amidated bile acids (e.g., taurocholic acid and glycocholic acid), and decreased clearance of indocyanine green in NASH subjects (Supplementary Reading List). Additionally, elevated plasma concentrations of acetaminophen-glucuronide and morphine-glucuronides were noted following parent drug administration in pediatric NASH patients compared to healthy subjects, possibly due to elevated MRP3/4 levels in NASH.[50,53]

Vildhede *et al.*[21] employed the LC-MS/MS based protein abundance data to assess the disease-induced changes in the *in vivo* hepatic drug disposition of transporter substrates (e.g., ^{99m}Tc-mebrofenin, rosuvastatin, morphine, and morphine glucuronides) by PBPK modeling and simulations.[21] A PBPK model incorporating NASH-mediated changes in transporter abundance and population demographics (i.e., obese population) captured the intravenous PK of ^{99m}Tc-mebrofenin (OATP1B/MRP2/MRP3 substrate) in NASH. The effect of NASH on the PK of morphine and its glucuronides was also modelled via PBPK.[21,54] The observed lack of a difference in OCT1 abundance in NASH liver relative to healthy tissues[21] was in agreement with the limited variability in morphine PK in NASH.[53] However, considerations of relative abundance/activity of MRP2 and MRP3 in NASH *versus* healthy, reasonably described an increase in the systemic exposure of morphine glucuronides in NASH. In addition to hepatic transporter and metabolizing enzyme changes, a better understanding of extrahepatic alterations of key protein activities in NASH would strengthen the PBPK approaches for this patient population.

Hepatitis C Virus Infection

Polypharmacy is a concern in DAA (Direct Acting Antiviral) drug treatment since comorbidities are common in HCV-infected patients. In addition, HCV DAA drugs with different mechanisms of action are often prescribed in combination to increase their efficacy, which further increases the risk of complex DDIs. Transporter abundance data for clean HCV samples are sparse due to co-morbidities with HCC, HIV (Human Immunodeficiency Virus) /AIDS or different stages of cirrhosis. As those co-morbidities and disease progression may regulate protein expression in the opposite direction of the HCV infection, interpretation of the proteomic data may become complicated (**Table S2**). Researchers that evaluated 'clean' HCV-infected tissue samples investigated the transporter mRNA changes in HCV samples compared to healthy control, but not the protein. Control samples for protein evaluations were from metastatic livers or from liver tissue of fibrosis or fatty livers, and only the mean/median expression data and the range were reported, not the individual data.[41,42,55] Hence, the effect of HCV infection on the individual transporter cannot be extracted from the currently reported data, partly due to the way the data have been published.

To our knowledge, PBPK models that account for disease-related transporter changes have not been developed to evaluate PK changes in HCV-infected patients. However, PBPK models for several anti-HCV drugs have been published recovering healthy volunteer data, for either BCRP perpetrators or for their metabolism pathways/corresponding DDI (references in Supplementary Reading List). Approved anti-HCV drugs with relevant transporter contributions and reported HI and/or RI clinical

data include glecaprevir, grazoprevir, pibrentasvir, and velpatasvir (**Table S1**), highlighting a need for further research on changes in transporter activity with progression of an HCV infection. For HBV (Hepatitis B Virus) and HIV, it has been shown that the virus itself interacts with certain transporters (e.g., NTCP, Supplementary Reading List). However, uptake of the HCV into hepatocytes is a complex multi-step process[56] and currently not linked to a specific hepatic drug transporter.

Cancer

Transporter expression in cancer cells has been extensively studied, particularly the expression of ABC transporters such as P-gp, with the aim to mitigate multidrug resistance.[57] Other transporters of interest in drug disposition were also found to have altered expression in tumor tissues. For example, expression levels of OATPs were reported to be altered in both hepatic and non-hepatic tumors; however, variant forms of some transporters (e.g., OATP1B3) were associated with different activity or functional role compared to OATPs in healthy livers.[49,58] As with other diseases, there is a growing interest in utilizing PBPK models coupled with quantitative proteomics to predict tumor penetration of therapeutics. Li et al. incorporated lower expression of P-gp/BCRP in glioblastoma samples[59] into PBPK models to capture the observed ribociclib concentrations in brain tumor, with the aim of increasing confidence in model-based comparison of CDK4/6 inhibitors for their ability to treat brain tumors.[60]

To predict the changes in the systemic exposure of transporter substrates in cancer patients, evaluation of transporter expression and function in drug eliminating organs (liver, kidney, intestine) in cancer patients is critical, regardless of whether tumor is present or not in these tissues. Kurzawski et al. recently evaluated transporter expression between healthy donor livers and non-tumorous tissue of metastatic livers.[61] While high variability was observed, a significant difference in protein abundance in whole tissue lysate was noted only for NTCP (higher in metastatic liver control) and P-gp (higher in healthy organ donor liver). Vasilogianni et al. also evaluated transporter expression in colorectal cancer patients with liver metastasis for both histologically normal and cancerous liver tissues, and reported downregulation of MRP2/3, OAT2/7 and OATP1B1/1B3/2B1.[62] Despite these changes, PBPK modeling predicted a relatively small impact on the systemic clearance for multiple transporter substrates (mainly OATP1B and P-gp substrates) (**Table 4**).

Systematic PK differences of transporter substrates between cancer patients and healthy subjects are not well studied, partly due to difficulties in conducting such studies and the lack of well-established probe substrates for transporters among small molecule anti-cancer agents.[63] One interesting clinical example is talazoparib, a dual P-gp and BCRP substrate that does not undergo extensive metabolism, unlike many other kinase inhibitors.[64] The PK of talazoparib was comparable between healthy and cancer patients (information on cancer type and stage not provided), suggesting that P-gp and BCRP activities in non-cancerous organs were not substantially altered in cancer patients. Information on transporter biomarkers in this diverse patient population is limited, with no clear trends (**Table 2**). For instance, trends in changes in baseline of the OATP1B1 biomarker CP-I were inconsistent, with studies reporting either higher or lower levels in some cancer populations relative to

healthy subjects. With improving life expectancy and greater likelihood of polypharmacy in the treatment of cancer, it is imperative to evaluate the function of transporters in cancer patients that might impact the disposition of medications used in this population using probe substrates or endogenous biomarkers, similar to the efforts being done for CYP enzymes.[65]

MODULATION IN TRANSPORTER EXPRESSION AND FUNCTION IN PEDIATRICS AND PREGNANCY

Age-dependent abundance of drug transporters and its impact on pharmacokinetics in the pediatric population

Due to ethical and logistical challenges in conducting clinical trials in children, the off-label use of medication in pediatric patients is prevalent, and can be up to 90% in neonates and infants.[6] In the intensive care units, infants and children are prescribed an average of 4 to 17 drugs, many of which are drug transporter substrates.[66] The Best Pharmaceuticals for Children Act 2002 and the Pediatric Research Equity Act 2003, which were strengthened by the Safety and Innovation Act 2012, have reaffirmed the FDA's commitment to conduct clinical studies in pediatric patients to increase the safety and efficacy of pediatric drug development. The empirical methods (e.g., body-weight or body-surface area-based methods) of pediatric dose extrapolation from adult data during first-in-child studies can lead to increased risk of therapeutic or toxic drug exposures in pediatric patients, especially young children, unless data on developmental biology are considered.

Developmental variability in drug transporter abundance and activity may alter drug exposure and clearance in children. For example, organic cation transporter 1 (OCT1) plays a critical role in the hepatic uptake of morphine, one of the top 5 drugs used in neonatal and pediatric intensive care units, prior to its glucuronidation.[66] Delayed developmental expression of hepatic OCT1 in neonates and infants, in conjunction with UGT2B7 is associated with a potential increase in plasma morphine concentration and a higher risk of respiratory depression.[67,68] The OCT1 expression increases by up to 5-fold after birth to reach adult levels within the first two years of life, whereas UGT2B7 increases gradually but dramatically (20-fold increase from neonates to adults). In the absence of clinical trials, FDA encourages use of PBPK models that incorporate age-dependent changes in drug disposition processes to predict drug exposure in pediatric patients. Specifically, under the Prescription Drug User Fee Act (PDUFA) VI, the FDA encourages use of PBPK modeling in the new drug application (NDA) review process, reflected in recently published examples[16] and increased number of PBPK analyses that were included in the NDA submissions to the FDA.[17]

Considering the increasing importance of pediatric PBPK modeling in drug development[8,16,17], it is encouraging to see rise in number of studies addressing the knowledge deficit with respect to ontogeny of drug metabolizing enzymes and transporters. The availability of LC-MS/MS proteomics techniques also played a crucial role in generating protein abundance data, which was not previously

possible for transmembrane proteins due to the lack of specific antibodies. **Figure 1** and **Table S3** provide a comprehensive summary of the currently available age-dependent protein abundance data of key clinically relevant transporters. While proteomics data on hepatic transporter ontogeny are available from multiple studies and cover younger populations, the ontogeny data on extrahepatic organs are still limited.[69,70] In particular, data for renal transporters in younger children and intestinal transporter data are only available either from individual studies or studies with limited sample sizes. These critical limitations should be considered when applying these data in pediatric PBPK models.

The hepatic protein abundance data for OATP1B1 show no age-dependent changes in the protein expression, especially when data are not stratified based on genotypes.[71] In particular, a clear trend of age-dependent increase between neonates and infants relative to the >1 year age group was observed when the analysis was limited to donors with the OATP1B1 reference genotype, *SLCO1B1*1*. Proteomic OATP1B3 LC-MS/MS abundance data are conflicting. One study showed no association of age with OATP1B3 protein expression[72], whereas, the second study[71] showed an age-dependent increase during the first year of life. Such discrepancy in ontogeny data is likely due to the limited number of tissue donors and high non-age related variability. The P-gp protein data show lower abundance in fetal, neonatal, or infant tissues as compared to adults, whereas MRP3 protein abundance levels in adolescents are lower than adults.[71] The postnatal OATP2B1, NTCP, MRP2, BCRP, and BSEP data are comparable across age based on protein abundance, whereas the protein ontogeny data for MRP1, MRP4, and MRP6 are limited. The ontogeny data for these transporters, including the available reported prenatal and mRNA data and the associated references, are discussed in more detail in the Supplementary text (Supplementary Reading List).

Studies characterizing the influence of pharmacogenetic variation the disposition of drug transporter substrates in children and adolescents are largely limited to OATP1B1-mediated statin PK and illustrate the challenges associated with extrapolation of adult data to pediatric patients (Table S4). Hedman *et al*[73] reported trends of lower systemic exposure in cardiac transplant recipients or patients with familial hypercholesterolemia and the c.521CC genotype (rs4149056), opposite to expected increase relative to patients with c.521TT genotypes based on adult data. However, it should be noted that the cardiac transplant patients were also receiving cyclosporin, a potent OATP1B1 inhibitor, which may have confounded the genotype-phenotype association. In contrast, in a genotype-stratified pharmacokinetic study in dyslipidemic children and adolescents Wagner *et al*[74] observed an approximately 2-fold increase in pravastatin systemic exposure, on average, per copy of c.521C allele, similar to the magnitude of effect reported in adults. Dose-corrected $AUC_{0\text{-}8}$ for simvastatin acid in hyperlipidemic children and adolescents was 6.3- and 2.5-fold greater in c.521CC (n=2) and c.521TC (n=15) cohorts relative to the c.521TT reference genotype (n=15), or approximately 2.5-fold higher per copy of the c.521T>C variant allele[75], which was greater than the magnitude of pharmacogenetic effect on $AUC_{0\text{-}\infty}$ ratios reported in adults[76]. A similar trend was observed for rosuvastatin.[77] For all three statins, the within-genotype variability (at least 10-fold) was considerably

greater than the ~2-fold between-genotype variability, indicating that additional factors beyond the c.521 SNP influence the dose-exposure relationship in children and adolescents.

Based on mRNA data, major intestinal transporters (P-gp, MRP2, and BCRP) do not show developmental expression when studied in neonates and infants compared to those in adults, whereas gene expression of pediatric OATP2B1 are suggested to be higher than in adults. However, a proteomics-based study shows age-dependent abundance of PEPT1, P-gp, BCRP, MRP2, PEPT1, P-gp, and BCRP (Table S3). In the case of clinically relevant renal transporters, OAT1 and OAT3, mRNA data suggest maturation of OATs in fetal and neonatal kidneys.[78] The data are supported by renal clearance of p-amino hippuric acid, which is ~5-fold lower at birth as compared to adults. However, the maturation of glomerular filtration also contributes to this large difference. The protein abundance of both OAT1 and OAT3 correlate with mRNA and activity data, however, the age-dependent changes are not conclusive after 1 year of age. Interestingly, high inter-protein correlations have been observed for OAT1 and OAT3 in both children and adults[69,71]. mRNA levels and protein abundance of another highly abundant uptake protein, OCT2 are also shown to be age-dependent during early age (age <2 years) as compared to adults. No age-dependent mRNA expression and protein abundance was detected for MRP2, MRP4, and MATE1 in preterm newborn to adults in kidneys. MATE2-K mRNA expression was significantly lower in newborns than that in adults, although the protein level was found to be stable from newborns to adults[9]. At the blood-brain barrier (BBB), P-gp expression in fetal or neonatal levels increase with age with up to ~2-fold higher levels in adults[79]. Another study utilizing semiquantitative immunostaining measurements confirmed increased protein expression of Pgp as well as BCRP with age, while MRP1 and MRP2 expression was higher in fetuses, neonates, and children, as compared to adults.[80]

Despite significant recent progress, there are several challenges that need to be considered when using reported transporter ontogeny data in PBPK modeling. Based on the existing data, it appears that the transporter expression demonstrates less marked age-dependent changes than observed with drug metabolizing enzymes. Nevertheless, this information is crucial for PBPK modeling when both drug metabolism and transport are involved in drug disposition. Non-monotonic developmental changes in an enzyme *versus* transporter of interest can lead to differential individual contributions of these processes relative to those in adults. Many drugs are metabolized by Phase I and Phase II pathways as well as eliminated by biliary excretion. For such types of drugs, equal contributions of metabolism (e.g., UGT1A1) and biliary efflux (e.g., BCRP) in elimination in adults may not necessarily translate to the same contribution in children, as illustrated in **Figure 2** using a hypothetical example. Therefore, it is important to understand ontogeny of each individual protein involved and whether differences in their individual development may shift the rate-determining process (metabolism *versus* transport) in children relative to adults. Because of non-monotonic changes in expression of transporter *versus* expression of enzymes with age, alteration of rate-determining processes is a likely scenario that would have implications on prediction of drug PK, DDI risk and the effect of genetic polymorphisms in certain pediatric age groups relative to adults. For example, if the uptake by

OATP1B1 transport (plus passive diffusion) prior to metabolism is the rate-determining step in adults, the moderate OATP1B1 ontogeny, the age-independent passive uptake and relatively marked CYP3A4 ontogeny is likely to result in different interplay and the contribution of individual processes in pediatric patients as compared to adults. Similar concepts have been explored for renal transporters, where the impact of transporter ontogeny on relative contribution of active renal secretion to renal clearance was investigated by simulation for drugs with different properties and across pediatric age groups. [81]

Pregnancy

Pregnancy results in physiological changes that may differ across the trimesters and lead to altered PK of certain drugs. Modulation in transporter and enzyme activities could be a major source of changes in drug disposition in pregnancy, although there are relatively limited clinical reports studying the actual changes in transporter abundances/activities.[82,83] Nevertheless, there is notable evidence implying altered transporter function in pregnancy. For example, increased renal secretion of digoxin was observed and calls for dose adjustments during pregnancy.[82,84,85] Although there was a correlation between glomerular filtration and digoxin renal clearance in pregnant women, it did not completely explain the increased digoxin renal clearance by nearly >2-fold, suggesting increased P-gp (and potentially OATP4C1 activity), in addition to glomerular filtration[84]. The renal secretory activity of OAT1/2/3 transporter probe substrate drugs in pregnant women[86] indicated >2-fold increase in drug exposure in certain trimesters for either OAT1, OAT2 or OAT3 relative to non-pregnant women, and indicated potential requirements for dose adjustment for these drugs. Such dose adjustments may be more relevant when considering the magnitude of the DDI, which may differ compared to non-pregnant individuals, as illustrated in the case of metabolism[20] Increased activity of renal OCT2/MATE1-2K transporters in the 2nd and 3rd trimesters in pregnant women also was suggested based on metformin as a probe drug, with up to 77% increase in the secretion clearance.[87,88] Although changes in protein abundances have been noted for certain hepatic transporters in pregnant mice, there have been limited reports regarding the clinical relevance of these changes.[89] Most recently, the protein abundance of OATP1B1 was suggested not to vary across trimesters based on data obtained using a novel liquid biopsy approach and quantification of this transporter using liver-derived small extracellular vesicles.[90]

Along with the PK changes in pregnancy or alterations in the maternal systemic and tissue drug concentrations, transporter expression at the placental barrier is an important factor determining the fetal drug exposure for certain compounds. OCT3, P-gp and BCRP protein abundance was suggested to change: P-gp, and BCRP levels were decreased, while the OCT3 expression increased with gestational age.[91,92] The activity of a particular transporter in the placental barrier can affect the transplacental passage of drugs and may result in sub-efficacious or toxic drug exposure to the fetus. PBPK modeling has also been used to predict the fetal drug exposures; however, the majority of these publications were based on data from *ex vivo* perfusion studies.[93] Explicit incorporation of transporter data is warranted for mechanistic extrapolations.[10,94]

PBPK MODELING OF TRANSPORTER SUBSTRATES IN DISEASE, PREGNANCY AND PEDIATRIC POPULATIONS

Table 4 summarizes recent progress and published examples of PBPK modeling for transporter substrates in specific populations, ranging from different disease populations (e.g., organ impairment, cancer and NASH) to pediatric populations and pregnant women. These examples of 'fit-for-purpose' PBPK models illustrate stepwise model development strategies, initial model verification against PK data in healthy/adult subjects and consideration of corresponding physiological differences in specific populations compared to the healthy/adult population. Case examples in **Table 4** emphasize the importance of careful scrutiny of the relevant enzyme/transporter alterations and other physiologic confounding factors for the appropriate model verification and application in specific/diseased population. When possible, virtual disease populations were informed by available transporter proteomics data (e.g., NASH[21]) to capture differential expression of these proteins in the disease. To bridge existing knowledge gaps in transporter expression/activity in certain diseases (e.g., renal impairment), PBPK models either relied on input from previous indirect analysis of clinical PK data in such patient cohorts (e.g., renal OATs[26]) or employed a reverse translation approach to calibrate the missing/uncertain system parameter(s) with late-stage clinical data.[28,39,95]

In addition to PBPK models for drug transporter substrates, PBPK modeling of endogenous transporter activity markers (such as creatinine) was used to denote altered activity of renal transporters in RI[30]. These mechanistic modeling exercises suggest that the activity of renal OAT1/2/3, OCT2 and MATE transporters decrease in a disproportionate manner relative to changes in GFR in RI, as opposed to the intact nephron hypothesis that assumes decrease in tubular secretion in proportion to GFR decline. However, the extent of disease-related modulation differed across transporters and disease severity. For instance, deterioration of OAT1/2/3 activity exceeded changes in GFR in severe CKD[31], whereas in the case of OCT2 and MATEs, the estimated decline in transporter activity was suggested to be smaller relative to changes in GFR.[30] Clinical consequences of disease-related modulation of specific transporter expression/activity will depend on the magnitude of these changes and the contribution of particular transport mechanism(s) to the systemic PK or tissue exposure (see examples in **Table 4**). PBPK modeling provides an excellent platform to explore these by sensitivity analyses for drugs with different contributions of active and passive processes, as highlighted for metformin (OCT2)[96] and ganciclovir (OATs)[26].

In addition to renal transporters, **Table 4** illustrates examples of PBPK models for drug substrates of hepatic and intestinal transporters in renal impairment, including repaglinide, statins, pemafibrate (OATP1B1) and digoxin/dabigatran (P-gp) amongst others, which can inform prospective PBPK applications on modulation of relevant transporter activity in disease populations. Transporter-enzyme interplay may further complicate the clinical PK interpretations for certain drugs, but equally in those cases PBPK modeling can be used to understand the impact of such interplay on the drug disposition

in renal and/or hepatic impaired population. The common feature is the requirement for robust mechanistic models and virtual disease populations to support evaluation of PK in untested scenarios (e.g., exploration of disease-drug-drug interactions). While the transporter expression data are still limited for "bottom-up" mechanistic modeling in organ impairment and other disease states, PBPK analyses of probe drugs with adequate verification offered insight into the functional modulation of the pathways. For instance, PBPK analyses of OATP1B drugs (**Table 4**) implied a decrease in hepatic uptake function by 30-60% in severe CKD patients. PBPK modeling of dabigatran etexilate/dabigatran implies up to 65% decrease in intestinal P-gp activity in severe RI, consistent with limited clinical data.[15] Additional clinical data are warranted to generalize these findings; however, these reports serve as informative case examples for future modeling.

Understanding transporter ontogeny and differences in developmental patterns between proteins is vital, as these may result in different sensitivity to transporter modulation in children of different ages relative to adults, as illustrated in examples discussed in Table 4 (morphine (OCT1), morphine-glucuronide (P-gp) and various substrates for renal OAT1/3). For certain age bands of the pediatric population (<1 year of age), the transporter ontogeny is an important consideration in the PBPK models for drug dose selection/adjustment and clinical implications such as transporter-mediated DDIs. It is evident that knowledge gaps in transporter developmental biology are still prominent and need to be addressed prior to developing such models, as reflected in limited PBPK case examples in the literature for transporter substrates in pediatrics.

Overview of regulatory PBPK applications related to transporters in organ impairment

Between January 2018 and December 2021, the U.S. FDA received a total number of 293 submissions, including 171 INDs and 122 NDAs/BLAs/ EUAs (Emergency Use Authorization) that contained PBPK modeling (one submission can include multiple purposes of PBPK application). Among these, 44% of submissions were intended for enzyme-mediated DDI evaluation, 17% for transporter-mediated DDI, and 12% were related to PK evaluations in pediatrics (**Figure 3**). If a submission involved both enzyme-mediated and transporter-mediated DDI, it was counted twice separately for the purpose of analysis. Although the total number of submissions in this period containing PBPK analyses has increased significantly compared to 2008-2017[97], the distribution of PBPK application areas has not changed significantly. A closer look at the transporter-mediated DDI evaluation illustrates that 59% of submissions was intended to evaluate the investigational drug as a perpetrator, whereas 27% evaluated the investigational drug as a substrate, and 14% were intended to evaluate the investigational drug both as a transporter perpetrator and substrate.

Among the submissions that included or proposed PBPK analysis to evaluate the effect of hepatic or renal impairment, transporters were not incorporated in a majority of the models. Letermovir, an OATP1B1 substrate, provides an example where the effect of HI on its PK was evaluated by PBPK modeling at different dose levels. Letermovir clearance involves saturable OATP1B1-mediated

uptake, and the PBPK sensitivity analysis suggested a greater increase in AUC at a lower dose level compared to at a higher dose assuming a disease-related decrease in OATP1B abundance. The PBPK analysis in conjunction with a dedicated HI PK study at a sub-therapeutic dose supported no dose adjustment for patients with mild to moderate HI at the clinically recommended dose, whereas letermovir is not recommended for patients with severe HI.[98] Another example noted in the recent submissions was asciminib, a substrate of P-gp and BCRP. In the asciminib PBPK model, BCRP was incorporated in the permeability-limited liver model to account for transporter-mediated biliary clearance. Due to the uncertainties in quantifying the contributions of various clearance pathways of asciminib, the model was inadequate to evaluate the impact of RI and HI on the PK of this drug.[99]

The examples above demonstrate a certain 'lag-time' between published cases/model explorations and application of those concepts with confidence for regulatory submissions and impact on labeling. One of the challenges in PBPK models for organ impairment for transporter substrates is that limited data are available to calibrate disease-related changes in transporter activity in corresponding models. This trend is in contrast to organ impairment models for CYP substrates where sizeable data allow more robust optimization of physiological/system parameters. As a result, there is a higher confidence in the use of PBPK modeling to predict the impact of RI for predominantly metabolized drugs (with varying contribution of renal clearance) and to support mild renal impairment study waivers or clinical study design, as concluded by industry consortium.[22] As the PBPK submissions for renal impairment remain low, the Agency is still accumulating experience in this area. With the known range of systemic predictive errors, a PBPK model could be useful in defining the inclusion and exclusion criteria, and dose adjustment if needed in phase 2/3 trials. To study the consequences of disease-related modulation of transporters, future PBPK analysis could be conducted initially for transporter substrates (such as listed in Table S1) with available hepatic or renal impairment clinical studies in order to gain confidence in the model structure and changes in the system data describing the disease progression and co-morbidities (e.g., NASH and/or obesity).

KNOWLEDGE GAPS AND CHALLENGES

Quantification of transporter protein abundance in the tissues of diseased and specific populations provides a valuable dataset to evaluate the change in transporter expression at a protein level relative to healthy subjects (**Table 3, Figure 1**). However, procedures of harvesting tissue samples and storage conditions vary across different laboratories. Similarly, detailed demographic information including medication use, disease conditions, and reason for death are not always reported and such information can influence transporter abundance data. Furthermore, there is a high inter-laboratory variability in transporter proteomic data even when the same tissue samples are tested, largely caused by the differences in sample preparation protocols, surrogate peptides with variable digestion efficiency, and LC-MS/MS methodologies.[43] These practical issues and technical challenges may confound the quality of transporter proteomic data and add uncertainty when applying these data for quantitative translation of PK in these populations. Furthermore, the correlation between protein

abundance and functional activity of transporters is not well-established in various diseases. Therefore, additional investigations are needed to understand whether disease-related changes in transport protein expression reflect alterations in functional activity. Similarly, transporter ontogeny data are based on protein abundance differences across age groups without considering any potential differences in the functional activity. Additional question is whether correlations in expression between different transport proteins established in healthy population remain also in disease. The same question is pertinent for adult *versus* pediatric patient or pregnant *versus* non-pregnant women. This information is critical for development of robust virtual disease/pediatric populations and corresponding PBPK models. As genetic polymorphisms can also affect transporter expression/activity, it is important to deconvolute individual effects of genotype and age-related changes in transporter abundance in pediatric samples. This complex interplay between transporter developmental changes and genotype will likely result in different impact of genetic polymorphism in younger children relative to adolescents and needs to be considered in data interpretation/extrapolation from one age group to another. In addition to expression, post-translational regulation could also alter functional activity of certain transporters[100], although clinical evidence and consideration of those changes in PBPK models is still lacking.

Clinical PK studies of transporter probe substrates in diseased and specific populations could be informative in understanding the changes in transporter function and their impact on PK (**Table 1**). However, lack of selectivity noted for many transporter probe substrates is a challenge with using such data, as absorption/elimination of many transporter clinical probes involves multiple transporters and/or complex interplay with metabolizing enzymes. Therefore, to accurately determine disease-related changes in transporter activity, it is essential to identify the rate-determining step and the contribution of transporters to disposition of these probe substrates, not only in healthy subjects, but also in diseased and specific populations. For instance, in pediatric populations, the rate-determining step may differ from adults because of different developmental rates of transporters and enzymes involved, which may confound interpretation of clinical data and derivation or transporter ontogeny functions from such data.

The PK studies following both intravenous and oral administration, or DDI studies with relatively selective inhibitors, will help deconvolute the change in intestinal and hepatic/renal transporters. However, such data are still very limited in these patient populations. Furthermore, lack of clinical studies with appropriate healthy subjects, patients with varied disease stages (e.g., mild, moderate, and severe organ impairment), and higher PK variability due to small sample sizes can also make the findings inconclusive. It is important to delineate the impact of changes in transporter activity from other disease-, and age-related physiological changes, such as alterations in plasma protein binding or GFR in CKD patients for transporter substrates that undergo renal elimination (e.g., metformin and dabigatran).

Table 4 highlights encouraging examples and best practices/strategies in development of robust PBPK models to capture changes in expression/activity of transporters in diseased and specific

populations. It is evident that confidence in the application of PBPK models in regulatory submissions for these populations is still limited, especially for transporter substrates. Successful application of PBPK modeling to these populations and evaluation of untested clinical scenarios (e.g., DDI risk) relies on an accurate determination of the physiological and biological changes, as well as changes in drug elimination pathways that can result in altered drug disposition in these populations compared with healthy/adult control subjects. It is apparent that certain virtual disease/specific populations require further refinement. For example, potential misspecification of virtual CKD patient populations has been noted when assessing the distributions of demographic and system data of virtual CKD subjects[26]. Despite these challenges in prospective use of PBPK modeling for specific populations, the way forward is to leverage the available clinical data for transporter substrates (including biomarkers) (**Tables 1 and 2**, **Table S1**) to gain confidence in the model structure and changes in the system data describing the disease progression. It is worth noting that changes in transporter activity in diseased and specific populations may not only affect systemic exposure of drugs, but also result in alterations of exposure in target organs such as the fetus or tumors, which may have potential efficacy and toxicological implications. As it is generally not feasible to directly measure such changes, mechanistic modeling and simulation represents an important translational tool to fill in these critical gaps. However, the validation of these model-based simulations is still challenging, as direct measurement of drug exposure in various tissues/fetuses and/or tumors in humans is generally not feasible. Availability of PD, toxicity, and/or tissue imaging data may refine some of the PBPK model parameters and transporter IVIVE to increase confidence in subsequent model applications.[3,101]

Compared to probe drugs, transporter biomarkers are novel and promising surrogate endogenous probes and have a potential to be used for studying transporter function *in vivo*, including in diseased and specific populations. Recently, some OATP1B biomarkers, such as CP-I have been explored to study changes in OATP1B activity and DDIs in CKD and cancer patients (**Table 2**). However, knowledge gaps in other specific populations and/or data for biomarkers for other transporters (e.g., renal transporters) remain. Despite increasing cumulative evidence to suggest the utility of biomarkers to assess transporter function and DDIs in healthy subjects[11,14], there are still knowledge gaps and additional considerations when interpreting biomarker data in diseased and specific populations. For example, organ impairment, ontogeny, and various diseases may also alter biomarker synthesis rates, and subsequently change the baseline level and systemic exposure of biomarkers in addition to potential modulation of transporter activity. Like probe drugs, the involvement of multiple transporters in the disposition and elimination of biomarkers may also confound data interpretation.

In addition to changes in transporter activity, some knowledge gaps and challenges may exist for other parameters in certain diseased and specific populations. For instance, there are limited data of measured $f_{u,p}$ values at different stages of CKD. Changes in drug plasma protein binding are attributed not only to changes in albumin concentrations, but also competition for albumin binding sites and potentially posttranslational modifications of the protein that may impact the PK of highly

bound drugs in CKD patients. In addition, recent studies highlighted potential alteration in albumin-facilitated OAT1 transport in CKD.[102] Changes in plasma albumin are also relevant for pediatric and pregnant populations. Currently, transporter abundance data are still not available for CKD patients due to limited access to tissue samples. The mechanisms for the changes in transporter activity caused by RI are complex and remain to be clarified. Accumulation of uremic toxins in CKD patients may impact transporter activity via inhibition, modification of albumin facilitated transport and/or regulation of transporter expression. However, *in vitro* and preclinical animal studies, and the translation of these data to clinic are still controversial or inconclusive. For the pediatric population, the ontogeny functions developed using a small number of samples could be misleading due to limited study power. This is particularly evident for neonatal subjects as none of the current transporter ontogeny functions indicate confidence in their use for this particular population within a PBPK framework. There is a scarcity of tissues or primary cells from pediatric donors, especially neonatal donors. As majority of the age-dependent changes in transporter and enzyme expression occur during the first few weeks of life, a thorough investigation using a large sample size of tissues or primary cells and more concerted efforts to collect and share these pediatric samples is required. High PK variability in the neonatal population due to variable gestational and postnatal age can also be a concern.

PERSPECTIVES AND FUTURE DIRECTIONS

Despite the complexity and knowledge gaps discussed above, cumulative studies suggested certain trends and changes in transporter abundance and/or activity in some diseased and specific populations. A decrease in OATP1B and likely intestinal P-gp, and renal OAT1/3 activity in CKD patients is evident (**Tables 1, 2 and 4**). Alteration in protein levels of multiple hepatic transporters (especially OATP1B and MRP3) is evident in NAFLD patients, like NASH, as well as OAT1/3 activity in pregnancy. Changes in transporter function may have clinical implications on systemic and likely tissue exposure of drugs. The potential for altered PK and likely safety/efficacy in these populations needs to be considered for drugs in which transporter-mediated uptake/efflux is a major contributor to absorption, distribution, and elimination and possible dose adjustment may be warranted especially for drugs with narrow therapeutic index. As a well-established tool, PBPK models (with verified system-, and drug-dependent parameters) have a capability for quantitative translation of changes in transporter activity in these populations.

To increase the confidence in prospective prediction of transporter-mediated PK, PD, and DDIs in diseased and specific populations, 1) continued efforts should be made in determining transporter protein abundance in the tissues from these populations using LC-MS/MS-based proteomic approaches. In addition to the standardization of tissue sample collection, LC-MS/MS quantification methods, understanding of the relationship between protein expression and function of clinically important transporters in healthy subjects, diseased, and specific populations will ultimately improve the translation of transporter proteomic data in PBPK models. 2) Recently, tissue-derived plasma small extracellular vesicles (sEVs) such as exosomes have emerged as a novel and promising tool in

determining the metabolic enzyme and transporter profile in human tissues[103-106]. The exosomes isolated from human plasma ('liquid biopsy') can provide a rich and informative dataset on protein expression of transporters in various tissues of healthy subjects, and diseased and specific populations, where the access of tissue samples is not feasible and/or limited. So far, a good correlation was demonstrated between mRNA in isolated human plasma exosomes and protein expression in the liver tissues for 12 key enzymes and 4 transporters (OATP1B1, P-gp, BCRP, and MRP2) in patients with liver cancer, in addition to some PD targets.[103] The studies using liver specific sEVs also suggested that hepatic OATP1B1 protein expression was not altered by pregnancy.[90] Data so far suggest that this technology holds the promise to fill in the knowledge gaps on the alterations of transporter function in different disease states and specific populations. However, isolation of tissue-specific sEVs for transporters expressed in multiple organs (e.g., liver and intestine) is required to increase robustness and applicability of this approach. Future research is necessary to evaluate the utility and limitations of liquid biopsy as a tool in studying transporter expression and function in disease. 3) There has been growing interest in exploring endogenous transporter biomarkers as surrogate clinical probes to study transporter function in diseased and specific populations. These efforts should be extended to other transporters (e.g., renal transporters), in addition to hepatic OATP1B, and multiple biomarkers that have distinct elimination profiles. A mechanistic understanding of the impact of disease states, ontogeny, and other pathophysiological related factors on biosynthesis, disposition, and elimination of biomarkers will aid in applying biomarker data to assess quantitatively the change of transporter activity in these patient populations. Establishing reference values of endogenous biomarkers for various populations can be important for the purpose of using biomarkers to estimate transporter activities for individual patients and dose optimization for transporter substrates. Furthermore, collaborative efforts to leverage liquid biopsy and biomarker data in the plasma samples obtained from the same individuals would help to establish the relationship between protein expression and functional activity, and whether/to what extent that relationship changes in the diseased and specific populations. 4) Generation of well-designed clinical data for relatively selective transporter probe substrates will greatly help to elucidate the change in transporter activity. These clinical data, integrated with transporter proteomic, plasma exosomes, and endogenous biomarker data will ultimately provide more definitive information on changes in transporter activity and allow refinement of virtual disease populations in PBPK models. 5) It is essential to continue development of PBPK models for specific populations and to improve their confidence to predict transporter related changes on PK, PD, and DDIs, with a possibility of open source models/ 'open science' approach for diseased or specific population models. Integration of multiomics, endogenous biomarkers and liquid biopsy data will help refinement of physiological/system parameters in PBPK models (e.g., transporter expression/activity) and development of robust disease/specific population models. Ultimately, this will increase the confidence and ability to simulate real-world combinations of covariates (e.g., advanced age, multiple diseases, dietary/environmental factors) and predict DDI risk in patients with multiple comorbidities.

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Table 1. Recent examples of the effects of diseases on pharmacokinetics of transporter substrates in humans

Disease state or patient population	Drug	Major transporters/ enzymes involved	Major elimination	N (patients, healthy subjects)	AUCR patients/healthy subjects[a]	Cmax ratios(CmaxR) patients/healthy subjects	V_d ratios patients/ healthy subjects[d]	References
Severe RI	Pitavastatin (10 µg P.O.)	OATP1B	Hepatic	(7, 6)	1.54 (0.90-2.63)[b, c]	1.64 (1.04-2.60)[b, c]	-	[15]
	Pitavastatin (4 mg P.O.)			(8, 8)	1.68 (1.08-2.60)[b, c]	1.46 (0.84, 2.49)[b, c]	-	[107]
	Rosuvastatin (50 µg P.O.)	OATP1B, BCRP, OATP2B1	Hepatic/renal	(7, 6)	1.49 (0.58-3.76)[b, c]	1.34 (0.61-3.01)[b, c]	-	[15]
	Atorvastatin (100 µg P.O.)	OATP1B, BCRP, P-gp, CYP3A	Hepatic	(7, 6)	1.94 (1.01-3.74)[b, c]	2.57 (1.16-5.73)[b, c]	-	[15]
	Dabigatran (375 µg DABE P.O.)	P-gp, CES1/2	Renal	(7, 6)	4.86 (2.56-9.26)[b, c]	1.71 (0.91-3.19)[b, c]	-	[15]
	Dabigatran (150 mg DABE P.O.)			(11, 6)	6.95 (3.90, 12.39)[b, c]	2.33 (1.38, 3.95)[b, c]		[108]
NAFLD No Fibrosis	Rosuvastatin (5 mg P.O.)	OATP1B, BCRP, OATP2B1	Hepatic/renal	(11, 12)	0.88	0.89	-	[109]
NAFLD-Fibrosis				(11, 12)	0.77	0.76	-	[109]
Steatosis	^{11}C-metformin (8 µg MAX IV bolus)	OCT1, OCT2, MATE1, MATE2K	Renal	(10, 4)	-	-	0.89	[110,111 112]
NASH				(8, 4)	-	-	0.79	[110-112]
NASH	^{99}mTc-mebrofenin	OATP1B1, OATP1B3, MRP2	Hepatic	(14, 7)	1.37	-	1.49	[52]

-: Not reported; * P< 0.05, **: P< 0.01
[a]: AUCR represents plasma AUC0-inf in patients relative to healthy control subjects conducted in the same studies
[b]: GMR (95 %CI)
[c]: AUCR and $C_{max}R$ corrected for plasma protein binding ($f_{u,\ p}$) in severe RI patients and healthy subjects. $f_{u,\ p}$ data in severe RI and healthy subjects were obtained from Tatosian et al.[15]
[d]: Vd obtained in compartmental model analysis with PET imaging data
BCRP: Breast Cancer Resistance Protein; CES, carboxyl esterase; MATE1/2K: Multi-drug and Toxin Extrusion Protein (Type 1/2K); NASH: Nonalcoholic Steatohepatitis; NAFLD: Nonalcoholic Fatty Liver Disease; OATP1B: Organic Anion Transporting Polypeptide 1B; OATP2B1: Organic Anion Transporting Polypeptide 2B1; OCT1: Organic Cation Transporter 1; P-gp: P-glycoprotein; RI, renal impairment.

Table 2. Summary of plasma exposure of OATP1B endogenous biomarkers CP-I and CP-III in specific/diseased population

Population/ Disease of interest	Subgroup	Mean Concentration [nM]		Reported metrics and derivation of concentration	N	Age		Race/ Ethnicity	Study location	Sex (Male/female)	Genotype	Reference
		CP-I	CP-III									
Reference (Meta-analysis)		0.685 (0.587-0.782)*	0.110 (0.096-0.124)*	-	-	-		-	-	-	-	-
Hyperlipidemic children	SLCO1B1 521TT	0.64**	-	AUC_{0-24h} (median) / 24h	13	Children		Not reported	USA	Not reported	SLCO1B1 521TT	[113]
	SLCO1B1 521TC	0.77**	-		13						SLCO1B1 521TC	
	SLCO1B1 521CC	1.1**	-		2						SLCO1B1 521CC	
CKD	Healthy	0.39	0.11	AUC_{0-24h} (GM) / 24h	6	58	(mean)	22 White, 9 Black/African American, 1 American Indian or Alaska Native; 20 Hispanic or Latino, 12 Not Hispanic or Latino	Not reported	21/11	Genotyped, numbers not reported	[15]
	Mild	0.48	0.10		7	65						
	Moderate	0.55	0.10		6	67						
	Severe	0.48	0.074		7	65						
	ESRD	0.8	-		6	57						
CKD	Healthy	0.69	-	Concentration (mean)	38	23	(mean)	Not reported	Japan	4/6	Not reported	[35]
	Stage 3-5 CKD	1.0	-			45				12/2		

	Stage 5D CKD	1.2	-			49				12/2		
ESRD (before and 90-days after transplantation)	Before	1.7	-	Concentration (mean)	13	50	(mean)	Not reported	Japan	12/1	Not reported	[36]
	After	0.99	-									
Rheumatoid Arthritis		0.97**	-	Concentration (mean)	37	64.5	(median)	Not reported	Japan	10/27	*15 carriers (n=13) and noncarriers (n=24)	[114]
NSCLC		2.21	0.448	AUC_{-3-7h} (mean) / 10h	10	48-75	(range)	Japanese	Japan	9/1	N = 7/3 for 521TT/TC	[115]
Metastatic solid tumor receiving irinotecan	SLCO1B1 521TT	1.6**	0.30**	Concentration (median)	13	68	(median)	Japanese	Japan	19/3	SLCO1B1 521TT	[116]
	SLCO1B1 521TC	2.3**	0.35**		4						SLCO1B1 521TC	
	SLCO1B1 521CC	3.6**	0.60**		3						SLCO1B1 521CC	
Locally advanced or metastatic HR+/HER2- BC		0.552	-	Baseline (model estimates)	21	59.8	(mean)	17 White, 1 Black/African American, 2 Asian, 2 Other or Missing	Global	Female only	Not reported	[117]

* Estimated mean and 95% confidence interval with meta-analysis (random effect model) of reported baseline CP-I levels in healthy or general population (see **Supplementary Method and Table S5** for detail**)**

** Values digitized from graphs.

CKD: chronic kidney disease; CP: coproporphyrin; ESRD: end-stage renal disease; HER: human epidermal growth factor receptor 2; HR: hormone receptor ; NSCLC: non-small cell lung cancer ; SLCO1B1: Solute Carrier Organic Anion Transporter Family Member 1B1.

Table 3: Comparison of transporter protein expression in livers of healthy subjects and in patients with liver diseases

Transporter	Ratios of hepatic transporter protein amount in diseased relative to healthy subjects (subject numbers in healthy, diseased population)										Unit of protein quantification	References
	Steatosis	NASH		Cirrhosis					Cancer			
			ALD-related	NAFLD-related	Biliary-related	Cancer-related	AIH-related	HCV-related	non-cancerous	cancerous		
OATP1B1	0.98 (50-52, 10)	0.69* (50-52, 10)	0.62*[d, f, j] (36, 27)					1.32[d, f, j] (36, 30), 1.37[d, f, j], (36, 30)	0.46*[d, f, j] (36, 33)	0.27*[d, f, j] (36, 8)	pmol/mg membrane protein	[21, 55, 118]
				1.21[g, h], 0.58[a, g, h], 1.14[b, g, h], 0.90[c, g, h] (14, 9)	0.73[g, h], 0.42[a, g, h], 1.00[b, g, h], 0.54[c, g, h] (14, 13)	0.47[g, h], 0.50[a, g, h], 0.91[b, g, h], 0.44[c, g, h] (14, 9)			0.71[g] (15, 16)	0.27[g] (15, 16)	pmol/mg microsomal protein	[42, 62]
			0.41*[d, f, j] (36, 27)					0.93[d, f, j] (36, 30), 0.96[d, f, j] (36, 30)	0.33*[d, f, j] (36, 33)	0.10*[d, f, j] (36, 8)	pmol/g liver	[55, 118]
			0.39*[e, h, i] (20, 20)		0.75[e, h, i] (20, 10), 0.74[e, h, i] (20, 6)		0.62[e, h, i] (20, 20)	0.49*[e, h, i] (20, 21)	1.51[e, i] (9,13)		fmol/mg whole liver protein	[41,61]
OATP1B3	0.77 (50-52, 10)	0.39* (50-52, 10)	0.46*[d, f, j] (36, 27)					0.73[d, f, j] (36, 30), 0.86[d, f, j] (36, 30)	0.39*[d, f, j] (36, 33)	0.26*[d, f, j] (36, 8)	pmol/mg membrane protein	[21, 55, 118]
			0.80*[d, f, j] (36, 27)					0.55*[d, f, j] (36, 30), 0.66*[d, f, j] (36, 30)	0.23*[d, f, j] (36, 33)		protein, pmol/g liver	[55, 118]
									0.98[g] (15, 16)	0.15[g] (15, 16)	pmol/mg microsomal protein	[62]
			0.21*[e, h, i] (20,		1.51[e, h, i] (20, 10),		1.26[e, h, i] (20,	0.42[e, h, i] (20, 21)	0.52[e, i] (9, 12)		fmol/mg whole liver protein	[41,61]

			20)		2.07 [e, h, i] (20, 6)		20)					
NTCP	0.83 (50-52, 10)	0.65* (50-52, 10)	1.02 [d, f, j] (36, 27)					0.67*, [d, f, j] (36, 30), 0.70*, [d, f, j] (36, 30)	3.00*, [d, f, j] (36, 33)	0.96 [d, f, j] (36, 8)	pmol/mg membrane protein	[21, 55, 118]
			0.65*, [d, f, j] (36, 27)					0.48*, [d, f, j] (36, 30), 0.50*, [d, f, j] (36, 30)	2.21*, [d, f, j] (36, 33)	0.41*, [d, f, j] (36, 8)	pmol/g liver	[55, 118]
			0.24 *, [e, h, i] (20, 20)		0.56 [e, h, i] (20, 10), 0.89 [e, h, i] (20, 6)		0.58 [e, h, i] (20, 20)	0.47 [e, h, i] (20, 21)	3.6*, [e, i] (9, 13)		fmol/mg whole liver protein	[41,61]
OATP2B1	0.89 (50-52, 10)	0.56* (50-52, 10)	1.15 [d, f, j] (36, 27)					1.51*, [d, f, j] (36, 30), 1.51*, [d, f, j] (36, 30)	0.89 [d, f, j] (36, 33)	0.56*, [d, f, j] (36, 8)	pmol/mg membrane protein	[21, 55, 118]
			0.78 [d, f, j] (36, 27)	0.30 *, [h] (14, 3)	0.50 [h] (14, 4)	0.33*, [h] (14, 4)		1.05 [d, f, j] (36, 30), 1.12 [d, f, j] (36, 30)	0.63*, [d, f, j] (36, 33)	0.25*, [d, f, j] (36, 8)	pmol/g liver	[55, 118, 119]
			0.27 *, [e, h, i] (20, 20)		0.40 [e, h, i] (20, 10), 0.80 [e, h, i] (20, 6)		0.47 [e, h, i] (20, 20)	0.26 *, [e, h, i] (20, 21)	2.55 [e, i] (9,13)		fmol/mg whole liver protein	[41,61]
OCT1				0.98 [g, h], 0.93 [b, g, h], 0.76 [c, g, h] (14, 9)	0.44 [g, h], 0.43 [b, g, h], 0.24 [c, g, h] (14, 13)	0.54 [g, h], 1.37 [b, g, h], 0.68 [c, g, h] (14, 9)					pmol/mg microsomal protein	[42]
	1.45 (50-52, 10)	0.95 (50-52, 10)	0.54*, [d, f, j] (36, 27)					0.63*, [d, f, j] (36, 30), 0.64*, [d, f, j] (36, 30)	0.33*, [d, f, j] (36, 33)	0.12*, [d, f, j] (36, 8)	pmol/mg membrane protein	[21, 55, 118]
			0.38*, [d, f, j] (36, 27)					0.45*, [d, f, j] (36, 30), 0.49*, [d, f, j] (36, 30)	0.26*, [d, f, j] (36, 33)		pmol/g liver	[55, 118]

			0.32 [e, h, i] (20, 20)		0.38 [*, e, h, i] (20, 10), 0.77 [e, h, i] (20, 6)		0.58 [e, h, i] (20, 20)	0.30 [*, e, h, i] (20, 21)	1.22 [e, i] (9,11)		fmol/mg whole liver protein	[41,61]
OAT2				2.23 [g, h], 1.50 [b, g, h], 1.22 [c, g, h] (14, 9)	0.77 [g, h], 1.00 [b, g, h], 0.56 [c, g, h] (14, 13)	0.67 [g, h], 1.88 [b, g, h], 0.89 [c, g, h] (14, 9)					pmol/mg microsomal protein	[42]
	0.86 (50-52, 10)	0.71* (50-52, 10)									pmol/mg membrane protein	[21]
				0.36 [h] (14, 3)	0.26 [h] (14, 4)	0.35 [h] (14, 4)					pmol/g liver	[119]
			0.26 [*, e, h, i] (20, 20)		0.74 [e, h, i] (20, 10), 1.14 [e, h, i] (20, 6)		1.05 [e, h, i] (20, 20)	0.54 [e, h, i] (20, 21)	0.90 [e, i] (9,12)		fmol/mg whole liver protein	[41,61]
BSEP				1.52 [g, h], 1.00 [a, g, h], 1.14 [b, g, h], 1.00 [c, g, h] (14, 9)	1.48 [g, h], 1.00 [a, g, h], 2.43 [b, g, h], 1.43 [c, g, h] (14, 13)	0.48 [g, h], 1.00 [a, g, h], 2.14 [b, g, h], 1.14 [c, g, h] (14, 9)					pmol/mg microsomal protein	[42]
	0.95 (50-52, 10)	0.82 (50-52, 10)	0.91 [d, f, j] (36, 27)					0.90 [d, f, j] (36, 30), 0.93 [d, f, j] (36, 30)	0.66* [d, f, j] (36, 33)	0.29* [d, f, j] (36, 8)	pmol/mg membrane protein	[21, 55, 118]
			0.57* [d, f, j] (36, 27)	0.28* [h] (14, 3)	0.62 [h] (14, 4)	0.38 [h] (14, 4)		0.63* [d, f, j] (36, 30), 0.67* [d, f, j] (36, 30)	0.46* [d, f, j] (36, 33)	0.12* [d, f, j] (36, 8)	pmol/g liver	[55, 118, 119]
			0.47 [e, h, i] (20, 20)		1.43 [e, h, i] (20, 10), 2.10 [e, h, i] (20, 6)		1.03 [e, h, i] (20, 20)	0.46* [e, h, i] (20, 21)	1.78 [e, i] (9, 13)		fmol/mg whole liver protein	[41,61]

MRP2	1.19 (50-52, 10)	0.71 (50-52, 10)	1.27[d, f, j] (36, 27)					0.95[d, f, j] (36, 30), 1.01[d, f, j] (36, 30)	2.25*[, d, f, j] (36, 33)	1.06[d, f, j] (36, 8)	pmol/mg membrane protein	[21,55, 118]
			0.79[d, f, j] (36, 27)	0.40[h] (14, 3)	0.33*[, h] (14, 4)	0.34*[, h] (14, 4)		0.66*[, d, f, j] (36, 30), 0.71*[, d, f, j] (36, 30)	1.60[d, f, j] (36, 33)	0.41*[, d, f, j] (36, 8)	pmol/g liver	[55, 118, 119]
			0.30*[, e, h, i] (20, 20)		0.18[e, h, i] (20, 10), 0.23[e, h, i] (20, 6)		0.18*[, e, h, i] (20, 20)	0.49[e, h, i] (20, 21)	2.93[e, i] (9,13)		fmol/mg whole liver protein	[41,61]
MRP3				3.44[g, h] (14, 9)	1.69[g, h] (14, 13)	0.81[g, h] (14, 9)					pmol/mg microsomal protein	[42]
	2.20* (50-52, 10)	1.73* (50-52, 10)	2.10*[, d, f, j] (36, 27)					1.79*[, d, f, j] (36, 30), 1.89*[, d, f, j] (36, 30)	0.74[d, f, j] (36, 33)	0.58[d, f, j] (36, 8)	pmol/mg membrane protein	[21,55, 118]
			1.38*[, d, f, j] (36, 27)	0.38[h] (14, 3)	0.56[h] (14, 4)	0.55[h] (14, 4)		1.21[d, f, j] (36, 30), 1.39[d, f, j] (36, 30)	0.52*[, d, f, j] (36, 33)	0.15*[, d, f, j] (36, 8)	pmol/g liver	[55, 118, 119]
			0.45[e, h, i] (20, 20)		0.51[e, h, i] (20, 10), 0.55[e, h, i] (20, 6)		0.59[e, h, i] (20, 20)	0.19[e, h, i] (20, 21)	0.60[e, i] (9,8)		fmol/mg whole liver protein	[41,61]
MDR1	1.83* (50-52, 10)	1.25 (50-52, 10)	1.75*[, d, f, j] (36, 27)					1.01[d, f, j] (36, 30), 1.07[d, f, j] (36, 30)	0.55*[, d, f, j] (36, 33)	0.89[d, f, j] (36, 8)	pmol/mg membrane protein	[21,55, 118]
			1.03[d, f, j] (36, 27)					0.65[d, f, j] (36, 30), 0.73*[, d, f, j] (36, 30)	0.36*[, d, f, j] (36, 33)	0.28*[, d, f, j] (36, 8)	pmol/g liver	[55, 118]
			1.39[e, h, i] (20, 20)		3.51*[, e, h, i] (20, 10),		4.10*[, e, h, i] (20, 20)	1.26[e, h, i] (20, 21)	0.18*[, e, i] (9, 10)		fmol/mg whole liver protein	[41,61]

					2.87*, e, h, i (20, 6)							
BCRP			0.76 d, f, j (36, 27)					1.32*, d, f, j (36, 30)			pmol/mg membrane protein	[118]
			0.55*, d, f, j (36, 27)					1.15 d, f, j (36, 30)			pmol/g liver	[118]
					1.78 e, h, i (20, 10)		1.33 e, h, i (20, 20)	0.40 e, h, i (20, 21)			fmol/mg whole liver protein	[41]
MATE1			1.69*, d, f, j (36, 27)					2.29*, d, f, j (36, 30), 1.93*, d, f, j (36, 30)	0.77 d, f, j (36, 33)	1.16 d, f, j (36, 8)	pmol/mg membrane protein	[55, 118]
			1.07 d, f, j (36, 27)					1.41*, d, f, j (36, 30), 1.55*, d, f, j (36, 30)		0.62 d, f, j (36, 8)	pmol/g liver	[55, 118]

***** Data are statistically significant different between healthy and diseased subjects.

Target proteomics quantification was used unless footnoted:

[a] High three (Hi3) ion intensity approach, [b] total protein approach (TPA) and [c] intensity-based absolute quantification (iBAQ) quantification.

[d] OATP1B1 SNPs information is available, and the reported membrane protein yield decreased in hepatocellular carcinoma (HCC) and cirrhosis compared to control samples in the reference papers.

[e] The transporter protein data in both control and diseased samples were reported as fmol/mg without unit definition in the figure legend or text.

[f] Data were extracted from reference papers using DigitizeIt software (Bormisoft, version 2.3.3).

[g] Mean values only were provided in the reference paper without statistical analysis.

[h] Control samples were excised from histologically normal areas from metastatic liver tissues.

[j] Control samples from Drozdzik et al.[41] are the same as non-cancerous tissue from metastatic livers[61]

[j] Same control and HCV cirrhotic samples were analyzed.

AIH: Autoimmune Hepatitis; ALD: Alcoholic Liver Disease; BCRP: Breast Cancer Resistance Protein; BSEP: Bile Salt Export Pump; HCV: Hepatitis C; MRP2: Multidrug Resistance Protein 2; MRP3: Multidrug Resistance Protein 2; MDR1: Multidrug Resistance P-glycoprotein; NASH: Nonalcoholic Steatohepatitis; NAFLD: Nonalcoholic Fatty Liver Disease; NTCP: Sodium/Taurocholate Co-transporting Polypeptide; OATP1B1: Organic Anion Transporting Polypeptide 1B1; OATP1B3: Organic Anion Transporting Polypeptide 1B3; OATP2B1: Organic Anion Transporting Polypeptide 2B1; OAT2: Organic Anion Transporter 2; OCT1: Organic Cation Transporter 1

Table 4. Recently published examples (2017- 2021) of PBPK modeling of organ impairment, cancer or NASH

Drug	Assumptions in the PBPK models to capture specific population	Step-wise PBPK model validation	Commentary and key PBPK model applications	Reference
Renal impairment – changes in renal transporters in PBPK models				
Creatinine	• Custom built reduced PBPK model (Matlab (R2017a) - mechanistic description of proximal tubule compartment • Incorporation of age related changes in creatinine synthesis • Modification of system parameters (e.g., volumes of proximal tubule compartments) proportional to the decline in GFR in RI • Consideration of membrane potential and bi-directionality in OCT2 transport • Different mechanisms of altered active secretion in CKD investigated, including decrease in OAT2, OCT2 and MATEs proportional to GFR (INH scenario[a]), and changes in transporter activity disproportionate to GFR i.e., non-INH scenario: - Decrease in OAT2 activity of 65-93% exceeded GFR changes - Disease-related change in OCT2 and MATEs was less than changes in the GFR	• Quantitative prediction of disease-related increase in serum creatinine (data from 64 G3 and G4 patients, 22-88 years) • Comparison of predicted CL_{cr}/GFR vs. GFR range	• Prediction of creatinine clearance in renal impairment and with OCT2/MATE inhibitor drugs • Quantitative prediction of interactions with famotidine, cimetidine and trimethoprim in patients for all transporter scenarios investigated - Intra-individual variability in serum considered in revised prediction limits - higher sensitivity to OCT2 / MATE inhibition in RI patients relative to healthy (non-INH assumption) • Under-prediction of cimetidine interactions highlights a need for a more mechanistic inhibitor model to account for its intra-tubular accumulation	[30]

Rivaroxaban	• PBPK model informed with *in vitro* transporter and metabolism data and human PK data • Mechanistic kidney model used in combination with Simcyp v17 default RI population models • Sensitivity analysis used to refine OAT3 $CL_{u,int}$ value to recover weighted mean CL_R value reported in healthy • Different mechanisms of altered active secretion modified in the RI population, including decrease in OAT3 and P-gp expression, and decrease in PTCPGK • INH assumptions applied to both scenarios[b] • The $f_{u,p}$ in RI assumed to be the same as in healthy ($f_{u,p}$ in healthy is 0.065)	• Simulations of rivaroxaban plasma PK and urinary excretion in healthy population • Prediction of DDI with CYP3A4/2J2 inhibitors (verapamil and ketoconazole)	• Prediction of clinically relevant disease-DDIs involving rivaroxaban • Mechanistic insight into OAT3 involvement in the renal elimination and DDIs of rivaroxaban • Inform dose adjustments to optimize rivaroxaban pharmacotherapy in atrial fibrillation	[120]
Valganciclovir - Ganciclovir	• Mechanistic kidney model used in combination with Simcyp (v 19.1) default RI population models • No differentiation in the contribution of individual OAT transporter (lumped OAT transporter clearance) • Different mechanisms of altered active secretion investigated in the RI population, including: i) Decrease in PTCPGK ii) Decrease in OATs and MATEs proportional to GFR (individually and combined) i) Disproportional decrease in OATs (attributed to the effect of uremic solutes) • The $f_{u,p}$ in RI model assumed the same as in healthy ($f_{u,p}$ in healthy is >0.9)	• PBPK model for healthy validated against independent clinical data set following oral administration of valganciclovir	• Prediction of changes in ganciclovir systemic exposure and renal clearance in RI patients • Evaluation of crystalluria risk in various clinical settings (old age, severe CKD and low fluid intake) by simulating ganciclovir medullary collecting duct concentrations • High crystalluria risk predicted for reduced urine flow (0.1mL/min) irrespective of underlying renal function	[26]

Renal impairment – changes in hepatic and intestinal transporters in PBPK models				
Repaglinide, Pitavastatin	• Disease-related changes protein binding accounted for by using measured $f_{u,p}$ in RI patients • 40-60% decrease in OATP1B1-mediated $CL_{int,T}$ obtained by reverse translation using *in vivo* observations in RI patients • Negligible disease-related change in CYP2C8 activity used in repaglinide model (based on analysis of CYP2C8 probes rosiglitazone and pioglitazone)	•PBPK model for healthy developed in Simcyp (v 16.1) validated against independent clinical data set, including OATP1B1 pharmacogenomic data in the same population and DDIs with OATP1B1 and CYP2C8 inhibitors	• Prediction of changes in systemic exposure and clearance in patients with moderate and severe renal impairment • Use of PBPK modeling to improve understanding of RI effect on OATP1B1 and/or CYP2C8 activity to guide the optimal use of non-renally eliminated drugs in RI patients	[95]
PF-04991532[c]	• PBPK model informed with *in vitro* hepatic and renal transport data and human PK data • Mechanistic kidney model and use of Simcyp v17 default RI population models • The $f_{u,p}$ in RI assumed to be the same as in healthy (0.14) • Estimated decrease in OATP1B and OAT3 activity of ~35% and ~80-90%, respectively to recover clinical data in moderate-severe RI • Metabolic clearance assigned to CYP3A4. Transporter-mediated uptake was rate-determining step for systemic PK. CYP3A4 abundance in RI population	• Base model verified across dose range (single and multiple dose) following oral administration. • Prediction of plasma conc. profiles and renal excretion in DDI study in healthy subjects with cyclosporine, an OATP1B inhibitor.	• Validate PBPK model to enable testing of complex scenarios like DDIs in organ impairment subjects, etc. • PBPK model applied to investigate disease-induced changes in liver (target tissue) exposure. • Model predicted marginally reduced PF-04991532 Kp_{uu} and increase in liver exposure in RI relative to healthy	[28]

	assumed to be the same as in healthy.			
Dabigatran etexilate (DABE)-dabigatran	• Clinical data used for refinement of DABE intestinal P-gp J_{max} in healthy • Renal excretion defined by renal clearance (from i.v. data in healthy) and modified in RI according to varying creatinine clearance (GFR cap applied to match clinical studies) • fu,p in RI assumed to be the same as in healthy (0.063)	• Base model applied for prediction of P-gp DDI with quinidine and verapamil in healthy subjects (Simcyp v 17.1) • Contribution of the inhibitor metabolite (nor-verapamil) considered, together with the impact of dose staggering • Prediction of dabigatran AUC and C_{max} after administration of DABE 150mg in mild, moderate and severe RI - Prediction of 10^{th}/90^{th} percentile of C_{trough} within the therapeutic range (28−210 ng/mL) as a criterion	• Underestimation of dabigatran concentration-time profiles in RI attributed to potential decrease in P-gp activity • Prediction of the DDI between DABE and the P-gp inhibitor verapamil in renal impairment population to support DABE dosing regimen and DDI risk evaluation	[39]
DABE, dabigatran and dabigatran glucuronide	• PBPK model of DABE, dabigatran, and dabigatran glucuronide (PK-Sim® and MoBi®, v8) extended from healthy to RI • Intestinal P-gp activity in severe RI reduced to 65%[d] of the baseline value in healthy adults • The $f_{u,p}$ values of DABE, dabigatran, and dabigatran glucuronide in RI predicted based on changes in albumin concentration relative to healthy[e] • Estimated 33% decline in UGT2B15 using clinical data in severe RI (values for GFR, $f_{u,p}$ and P-gp activity fixed as stated above)	• Model in heathy validated for prediction of rifampicin and clarithromycin DDI with DABE • RI model validated against independent clinical data to the ones used for model development - prediction of oral PK of dabigatran and total dabigatran (unconjugated dabigatran and glucuronide)	• Simulated impact of altered P-gp and UGT2B15 activity in the RI on dabigatran PK after 150 mg sd or 75mg bid • Extension of the model to PBPK/PD to predict the effects of idarucizumab on the PD of dabigatran in RI patients	[121,122]

Pemafibrate	• PBPK model (Simcyp v18) informed with *in vitro* transporter and metabolism data • Clinical PK data used for refinement of P_{eff} and $CLint_{,OATP1B1}$ in PBPK model for healthy • Decrease in OATP1B1 activity to 47% and 69% in Child-Pugh A and B population, respectively, compared to healthy subjects • No modifications in OATP1B1 activity was considered in renal impairment virtual population	• Model in heathy validated for prediction of pemafibrate PK in Japanese and DDI with rifampicin DDI	• Prediction of pemafibrate DDIs with OATP1B1 inhibitors (rifampicin, sacubitril) in both hepatic and renal impairment patients	[123]
Other disease populations				
Repaglinide, atorvastatin, dabigatran etexilate, valsartan, rosuvastatin, pravastatin, digoxin	• Compounds files available in Simcyp compound library (V20.1) were utilized • Measured abundance of drug transporters in histologically normal and cancerous tissues of colorectal cancer patients with liver metastasis (CRLM) were used • Disease-related differences in MPPGL were also incorporated • Two simulations were performed for CRLM– assuming either (1) the whole liver is histologically normal or (2) the whole liver is cancerous	• Clinical PK data in CRLM not available for validation	• For many drugs investigated in this dataset, substantial difference in the ratio of CL observed depending on which group is used as a reference, healthy population *versus* CRLM with histologically normal liver tissue • PBPK model predicted relatively small (<2-fold) difference in CL with liver metastasis for majority of transporter substrates • Further validation with clinical PK data in CRLM subjects is needed to confirm	[62]

			findings	
Ribociclib, palbociclib, abemaciclib	• 4-compartment permeability-limited brain model (4Brain, Simcyp v 18.1) with custom modification was used • Varying brain pH based on electrode measurement and pH-dependent apparent permeability used • Proteomic data on decrease in P-gp and BCRP abundance in brain microvessels in glioblastomas incorporated in the model • RAF value for P-gp-mediated active influx at the blood-CSF barrier was assigned and verified by the observed clinical CSF data of ribociclib	• Ribociclib concentrations (plasma, CSF, and brain) were captured well, after incorporating pH difference and/or decrease in P-gp expression	• The developed model was used to predict differences in brain penetration across the three CDK4/6 inhibitors and possible dose optimizations	[60]
Morphine, morphine-glucuronides, ^{99m}Tc-mebrofenin, rosuvastatin	• Full PBPK models (Simcyp v 18.1) accounting for permeability-limited hepatic disposition • Hepatic processes in the model informed with *in vitro* transporter data • Transporter protein abundance (LC-MS/MS) measured in steatosis/NASH liver samples were used to model PK in disease state	• PBPK models verified with PK data in healthy subjects. In case of mebrofenin, PBPK model was also verified using data from DDI study with OATP1B inhibitor (ritonavir) • Model accounting for relative abundance (NASH/healthy) for OATP1B, OCT1, MRP2/3 well predicted PK changes of these drugs and/or glucuronide metabolites in NASH	• A proteomic informed PBPK approach was developed to predict PK changes in NAFLD/NASH subjects for transporter substrates • Potential changes in renal and intestinal transport activity not accounted, and needs further work	[54] [21]
Pediatric population				

Morphine	• Study updated morphine PBPK model for neonates and infants described in (Emoto 2018) • Morphine PBPK model (Simcyp v 16.1) was extended to neonates (pre- and full-term) i) UGT2B7 and OCT1 ontogeny functions in neonates applied ii)OCT1 genotype- dependent kinetic parameters used in the model	• PBPK model was verified with morphine PK data from 83 OCT1-genotyped neonatal patients	• The PBPK model was used to assess the impact of OCT1 ontogeny and genetic variation on morphine PK disposition in neonatal patients • A retrospective power analysis using the allometrically standardized morphine clearance values simulated by the PBPK model used to determine the adequate patient sample size to guide the design of prospective studies	[68]
Morphine-morphine glucuronide	• Four-compartment brain PBPK/PD model developed in R (Version 3.6.2) to predict morphine brain disposition and analgesic effect (accounted also for active 6-glucuronide metabolite) • Pgp-mediated brain efflux implemented by *in vitro - in vivo* extrapolation of transporter kinetic data (MDCK-Pgp) and accounting for developmental Pgp expression in the brain • Morphine clearance was adjusted to observed plasma PK in adults, children and infants • PD model incorporated binding of morphine and morphine-6-glucuronide to the u opioid and used PBPK model simulated unbound brain concentrations	• Model simulations were verified with morphine and morphine-6 glucuronide plasma, brain extracellular fluid (ECF) and cerebrospinal fluid (CSF) PK data in adults and children (1-16 years) patients with differing underlying diseases • PBPK/PD model was successfully verified with analgesic data from adults and children	•The PBPK model prospectively predicted CSF concentrations for 0–11 months postnatal age • Simulated neonatal plasma data were compared with plasma PK from another study due to lack of matched samples • Analgesic effect-time profiles were under-predicted in neonates; altered morphine PD relative to adults and children was proposed (model only implemented age-related differences in P-gp activity)	[124]

Clavulanic acid Amoxicillin Cefazolin Piperacillin	• A combined popPK and PBPK modeling approach (NONMEM v7.3 and R v3.5) was used to estimate the *in vivo* functional ontogeny of OAT1 and OAT3 • OAT1/3 ontogeny was estimated using PK data for clavulanic acid (OAT1) and amoxicillin (OAT3) in intensive care pediatric patients (1 month - 15 years; median age of 2.6 years)	• The estimated ontogeny of OAT1 and OAT3 were prospectively applied for prediction of renal clearance of piperacillin and cefazolin • PBPK model with OAT ontogeny correctly predicted piperacillin CLr in age range of 2.5 months–15 years and cefazolin CLr in neonates	• OAT1/3 mediated intrinsic clearance and the resultant estimated ontogeny was described by a sigmoidal function and the OAT1/3 ontogeny activity were estimated as reaching the half of adult level around 7 months of age	[125]

[a]INH - intact nephron hypothesis

[b] Decrease in OAT3 and P-gp expression was represented in mechanistic kidney model (MechKiM) by assigning relative abundances for the OAT3 and P-gp transporters in kidney in the "poor transporter" (PT) phenotype as a proportion of the "extensive transporter" phenotype value of 1, as reported previously [34]. Changes were applied equally to each of the sub-region of the proximal tubule.

[c]Liver-targeted glucokinase activator

[d]Decrease in intestinal P-gp activity in severe RI is based on data in rat

[e]$f_{u,p}$ values of DABE, dabigatran, and dabigatran glucuronide in severe RI were 8.4% (7%), 68.9% (65%) and 68.9% (65%), respectively. Numbers in brackets indicate the corresponding $f_{u,p}$ values in healthy subjects.

[f] The portal liver blood flow was reduced to 40 and 36% of the healthy value for mild and moderate HI, respectively. The liver volume fraction was reduced to 81 and 65% for mild and moderate HI relative to the volume of healthy subjects.

BCRP: Breast Cancer Resistance Protein; CL_{cr} - creatinine clearance; G3 – moderate renal impairment; G4 – severe renal impairment; NASH: Nonalcoholic Steatohepatitis; MPPGL - Microsomal Protein Per Gram of Liver; MRP2: Multidrug Resistance Protein 2; NAFLD: Nonalcoholic Fatty Liver Disease; NTCP: Sodium/Taurocholate Co-transporting Polypeptide; OATP1B1: Organic Anion Transporting Polypeptide 1B1; OAT1/3: Organic Anion Transporter 1/3; OCT1: Organic Cation Transporter 1; PBPK: physiologically-based pharmacokinetic; P_{eff} ; effective permeability; PTCPGK: Proximal Tubular Cells Per Gram of Kidney; UGT2B15: Uridine Diphosphate (UDP) Glucuronosyltransferase 2B15

REFERENCES

1. International Transporter C, Giacomini KM, Huang SM, et al. Membrane transporters in drug development. *Nat Rev Drug Discov.* 2010;9(3):215-236.
2. Chu X, Korzekwa K, Elsby R, et al. Intracellular drug concentrations and transporters: measurement, modeling, and implications for the liver. *Clin Pharmacol Ther.* 2013;94(1):126-141.
3. Guo Y, Chu X, Parrott NJ, et al. Advancing Predictions of Tissue and Intracellular Drug Concentrations Using In Vitro, Imaging and Physiologically Based Pharmacokinetic Modeling Approaches. *Clin Pharmacol Ther.* 2018;104(5):865-889.
4. Evers R, Piquette-Miller M, Polli JW, et al. Disease-Associated Changes in Drug Transporters May Impact the Pharmacokinetics and/or Toxicity of Drugs: A White Paper From the International Transporter Consortium. *Clin Pharmacol Ther.* 2018;104(5):900-915.
5. FDA. Pharmacokinetics in Patients with Impaired Renal Function — Study Design, Data Analysis, and Impact on Dosing and Labeling. 2020.
6. FDA. General Clinical Pharmacology Considerations for Pediatric Studies for Drugs and Biological Products. 2014.
7. Brouwer KL, Aleksunes LM, Brandys B, et al. Human Ontogeny of Drug Transporters: Review and Recommendations of the Pediatric Transporter Working Group. *Clin Pharmacol Ther.* 2015;98(3):266-287.
8. Cheung KWK, van Groen BD, Burckart GJ, Zhang L, de Wildt SN, Huang SM. Incorporating Ontogeny in Physiologically Based Pharmacokinetic Modeling to Improve Pediatric Drug Development: What We Know About Developmental Changes in Membrane Transporters. *J Clin Pharmacol.* 2019;59 Suppl 1:S56-S69.
9. Cheung KWK, van Groen BD, Spaans E, et al. A Comprehensive Analysis of Ontogeny of Renal Drug Transporters: mRNA Analyses, Quantitative Proteomics, and Localization. *Clin Pharmacol Ther.* 2019;106(5):1083-1092.
10. Dallmann A, Liu XI, Burckart GJ, van den Anker J. Drug Transporters Expressed in the Human Placenta and Models for Studying Maternal-Fetal Drug Transfer. *J Clin Pharmacol.* 2019;59 Suppl 1:S70-S81.
11. Chu X, Liao M, Shen H, et al. Clinical Probes and Endogenous Biomarkers as Substrates for Transporter Drug-Drug Interaction Evaluation: Perspectives From the International Transporter Consortium. *Clin Pharmacol Ther.* 2018;104(5):836-864.
12. Mochizuki T, Zamek-Gliszczynski MJ, Yoshida K, et al. Effect of Cyclosporin A and Impact of Dose Staggering on OATP1B1/1B3 Endogenous Substrates and Drug Probes for Assessing Clinical Drug Interactions. *Clin Pharmacol Ther.* 2022.
13. Takita H, Barnett S, Zhang Y, et al. PBPK Model of Coproporphyrin I: Evaluation of the Impact of SLCO1B1 Genotype, Ethnicity, and Sex on its Inter-Individual Variability. *CPT Pharmacometrics Syst Pharmacol.* 2021;10(2):137-147.
14. Willemin ME, Van Der Made TK, Pijpers I, et al. Clinical Investigation on Endogenous Biomarkers to Predict Strong OAT-Mediated Drug-Drug Interactions. *Clin Pharmacokinet.* 2021;60(9):1187-1199.
15. Tatosian DA, Yee KL, Zhang Z, et al. A Microdose Cocktail to Evaluate Drug Interactions in Patients with Renal Impairment. *Clin Pharmacol Ther.* 2021;109(2):403-415.
16. Cleary Y, Gertz M, Grimsey P, et al. Model-Based Drug-Drug Interaction Extrapolation Strategy From Adults to Children: Risdiplam in Pediatric Patients With Spinal Muscular Atrophy. *Clin Pharmacol Ther.* 2021;110(6):1547-1557.
17. Zhang X, Yang Y, Grimstein M, et al. Application of PBPK Modeling and Simulation for Regulatory Decision Making and Its Impact on US Prescribing Information: An Update on the 2018-2019 Submissions to the US FDA's Office of Clinical Pharmacology. *J Clin Pharmacol.* 2020;60 Suppl 1:S160-S178.
18. Shebley M, Sandhu P, Emami Riedmaier A, et al. Physiologically Based Pharmacokinetic Model Qualification and Reporting Procedures for Regulatory Submissions: A Consortium Perspective. *Clin Pharmacol Ther.* 2018;104(1):88-110.
19. Frechen S, Rostami-Hodjegan A. Quality Assurance of PBPK Modeling Platforms and Guidance on Building, Evaluating, Verifying and Applying PBPK Models Prudently under the Umbrella of Qualification: Why, When, What, How and By Whom? *Pharm Res.* 2022.

20. Sychterz C, Galetin A, Taskar KS. When special populations intersect with drug-drug interactions: Application of physiologically-based pharmacokinetic modeling in pregnant populations. *Biopharm Drug Dispos.* 2021;42(4):160-177.
21. Vildhede A, Kimoto E, Pelis RM, Rodrigues AD, Varma MVS. Quantitative Proteomics and Mechanistic Modeling of Transporter-Mediated Disposition in Nonalcoholic Fatty Liver Disease. *Clin Pharmacol Ther.* 2020;107(5):1128-1137.
22. Heimbach T, Chen Y, Chen J, et al. Physiologically-Based Pharmacokinetic Modeling in Renal and Hepatic Impairment Populations: A Pharmaceutical Industry Perspective. *Clin Pharmacol Ther.* 2021;110(2):297-310.
23. Taskar KS, Pilla Reddy V, Burt H, et al. Physiologically-Based Pharmacokinetic Models for Evaluating Membrane Transporter Mediated Drug-Drug Interactions: Current Capabilities, Case Studies, Future Opportunities, and Recommendations. *Clin Pharmacol Ther.* 2020;107(5):1082-1115.
24. Tan ML, Yoshida K, Zhao P, et al. Effect of Chronic Kidney Disease on Nonrenal Elimination Pathways: A Systematic Assessment of CYP1A2, CYP2C8, CYP2C9, CYP2C19, and OATP. *Clin Pharmacol Ther.* 2018;103(5):854-867.
25. Yoshida K, Sun B, Zhang L, et al. Systematic and quantitative assessment of the effect of chronic kidney disease on CYP2D6 and CYP3A4/5. *Clin Pharmacol Ther.* 2016;100(1):75-87.
26. Scotcher D, Galetin A. PBPK Simulation-Based Evaluation of Ganciclovir Crystalluria Risk Factors: Effect of Renal Impairment, Old Age, and Low Fluid Intake. *AAPS J.* 2021;24(1):13.
27. Yeung CK, Yoshida K, Kusama M, et al. Organ Impairment-Drug-Drug Interaction Database: A Tool for Evaluating the Impact of Renal or Hepatic Impairment and Pharmacologic Inhibition on the Systemic Exposure of Drugs. *CPT Pharmacometrics Syst Pharmacol.* 2015;4(8):489-494.
28. Bergman A, Bi YA, Mathialagan S, et al. Effect of Hepatic Organic Anion-Transporting Polypeptide 1B Inhibition and Chronic Kidney Disease on the Pharmacokinetics of a Liver-Targeted Glucokinase Activator: A Model-Based Evaluation. *Clin Pharmacol Ther.* 2019;106(4):792-802.
29. Hsueh CH, Yoshida K, Zhao P, et al. Identification and Quantitative Assessment of Uremic Solutes as Inhibitors of Renal Organic Anion Transporters, OAT1 and OAT3. *Mol Pharm.* 2016;13(9):3130-3140.
30. Takita H, Scotcher D, Chinnadurai R, Kalra PA, Galetin A. Physiologically-Based Pharmacokinetic Modelling of Creatinine-Drug Interactions in the Chronic Kidney Disease Population. *CPT Pharmacometrics Syst Pharmacol.* 2020;9(12):695-706.
31. Tan S, Scotcher D, Galetin A. Uncovering the disproportional impact of chronic kidney disease on the renal secretion of drugs via organic anion transporters (OAT) 1 and 3. *Clinical Pharmacology & Therapeutics.* 2022;Suppl 1(S67).
32. Cheung KWK, Hsueh CH, Zhao P, et al. The Effect of Uremic Solutes on the Organic Cation Transporter 2. *J Pharm Sci.* 2017;106(9):2551-2557.
33. Naud J, Michaud J, Beauchemin S, et al. Effects of chronic renal failure on kidney drug transporters and cytochrome P450 in rats. *Drug Metab Dispos.* 2011;39(8):1363-1369.
34. Scotcher D, Jones CR, Galetin A, Rostami-Hodjegan A. Delineating the Role of Various Factors in Renal Disposition of Digoxin through Application of Physiologically Based Kidney Model to Renal Impairment Populations. *J Pharmacol Exp Ther.* 2017;360(3):484-495.
35. Suzuki Y, Sasamoto Y, Yoshijima C, et al. Simultaneous quantification of coproporphyrin-I and 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid in human plasma using ultra-high performance liquid chromatography coupled to tandem mass spectrometry. *J Pharm Biomed Anal.* 2020;184:113202.
36. Suzuki Y, Ono H, Tanaka R, et al. Recovery of OATP1B Activity after Living Kidney Transplantation in Patients with End-Stage Renal Disease. *Pharm Res.* 2019;36(4):59.
37. Chu X, Galetin A, Zamek-Gliszczynski MJ, Zhang L, Tweedie DJ, International Transporter C. Dabigatran Etexilate and Digoxin: Comparison as Clinical Probe Substrates for Evaluation of P-gp Inhibition. *Clin Pharmacol Ther.* 2018;104(5):788-792.
38. Lang J, Vincent L, Chenel M, Ogungbenro K, Galetin A. Reduced physiologically-based pharmacokinetic model of dabigatran etexilate-dabigatran and its application for prediction of intestinal P-gp-mediated drug-drug interactions. *Eur J Pharm Sci.* 2021;165:105932.
39. Doki K, Neuhoff S, Rostami-Hodjegan A, Homma M. Assessing Potential Drug-Drug Interactions Between Dabigatran Etexilate and a P-Glycoprotein Inhibitor in Renal Impairment Populations Using Physiologically Based Pharmacokinetic Modeling. *CPT Pharmacometrics Syst Pharmacol.* 2019;8(2):118-126.

40. Schuppan D, Afdhal NH. Liver cirrhosis. *Lancet.* 2008;371(9615):838-851.
41. Drozdzik M, Szelag-Pieniek S, Post M, et al. Protein Abundance of Hepatic Drug Transporters in Patients With Different Forms of Liver Damage. *Clin Pharmacol Ther.* 2020;107(5):1138-1148.
42. El-Khateeb E, Al-Majdoub ZM, Rostami-Hodjegan A, Barber J, Achour B. Proteomic Quantification of Changes in Abundance of Drug-Metabolizing Enzymes and Drug Transporters in Human Liver Cirrhosis: Different Methods, Similar Outcomes. *Drug Metab Dispos.* 2021;49(8):610-618.
43. Prasad B, Achour B, Artursson P, et al. Toward a Consensus on Applying Quantitative Liquid Chromatography-Tandem Mass Spectrometry Proteomics in Translational Pharmacology Research: A White Paper. *Clin Pharmacol Ther.* 2019;106(3):525-543.
44. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol.* 2018;15(1):11-20.
45. Haque M, Sanyal AJ. The metabolic abnormalities associated with non-alcoholic fatty liver disease. *Best Pract Res Clin Gastroenterol.* 2002;16(5):709-731.
46. Murphy WA, Adiwidjaja J, Sjostedt N, et al. Considerations for Physiologically Based Modeling in Liver Disease: From Nonalcoholic Fatty Liver (NAFL) to Nonalcoholic Steatohepatitis (NASH). *Clin Pharmacol Ther.* 2022.
47. Lake AD, Novak P, Fisher CD, et al. Analysis of global and absorption, distribution, metabolism, and elimination gene expression in the progressive stages of human nonalcoholic fatty liver disease. *Drug Metab Dispos.* 2011;39(10):1954-1960.
48. Hardwick RN, Fisher CD, Canet MJ, Scheffer GL, Cherrington NJ. Variations in ATP-binding cassette transporter regulation during the progression of human nonalcoholic fatty liver disease. *Drug Metab Dispos.* 2011;39(12):2395-2402.
49. Thakkar N, Slizgi JR, Brouwer KLR. Effect of Liver Disease on Hepatic Transporter Expression and Function. *J Pharm Sci.* 2017;106(9):2282-2294.
50. Canet MJ, Merrell MD, Hardwick RN, et al. Altered regulation of hepatic efflux transporters disrupts acetaminophen disposition in pediatric nonalcoholic steatohepatitis. *Drug Metab Dispos.* 2015;43(6):829-835.
51. Clarke JD, Novak P, Lake AD, Hardwick RN, Cherrington NJ. Impaired N-linked glycosylation of uptake and efflux transporters in human non-alcoholic fatty liver disease. *Liver Int.* 2017;37(7):1074-1081.
52. Ali I, Slizgi JR, Kaullen JD, et al. Transporter-Mediated Alterations in Patients With NASH Increase Systemic and Hepatic Exposure to an OATP and MRP2 Substrate. *Clin Pharmacol Ther.* 2017;104(4):749 - 756.
53. Ferslew BC, Xie G, Johnston CK, et al. Altered Bile Acid Metabolome in Patients with Nonalcoholic Steatohepatitis. *Dig Dis Sci.* 2015;60(11):3318-3328.
54. Sjostedt N, Neuhoff S, Brouwer KLR. Physiologically-Based Pharmacokinetic Model of Morphine and Morphine-3-Glucuronide in Nonalcoholic Steatohepatitis. *Clin Pharmacol Ther.* 2021;109(3):676-687.
55. Billington S, Ray AS, Salphati L, et al. Transporter Expression in Noncancerous and Cancerous Liver Tissue from Donors with Hepatocellular Carcinoma and Chronic Hepatitis C Infection Quantified by LC-MS/MS Proteomics. *Drug Metab Dispos.* 2018;46(2):189-196.
56. Zhu YZ, Qian XJ, Zhao P, Qi ZT. How hepatitis C virus invades hepatocytes: the mystery of viral entry. *World J Gastroenterol.* 2014;20(13):3457-3467.
57. Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, Gottesman MM. Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer.* 2018;18(7):452-464.
58. Schulte RR, Ho RH. Organic Anion Transporting Polypeptides: Emerging Roles in Cancer Pharmacology. *Mol Pharmacol.* 2019;95(5):490-506.
59. Bao X, Wu J, Xie Y, et al. Protein Expression and Functional Relevance of Efflux and Uptake Drug Transporters at the Blood-Brain Barrier of Human Brain and Glioblastoma. *Clin Pharmacol Ther.* 2020;107(5):1116-1127.
60. Li J, Jiang J, Wu J, Bao X, Sanai N. Physiologically Based Pharmacokinetic Modeling of Central Nervous System Pharmacokinetics of CDK4/6 Inhibitors to Guide Selection of Drug and Dosing Regimen for Brain Cancer Treatment. *Clin Pharmacol Ther.* 2021;109(2):494-506.
61. Kurzawski M, Szelag-Pieniek S, Lapczuk-Romanska J, et al. The reference liver - ABC and SLC drug transporters in healthy donor and metastatic livers. *Pharmacol Rep.* 2019;71(4):738-745.

62. Vasilogianni AM, Al-Majdoub ZM, Achour B, Peters SA, Rostami-Hodjegan A, Barber J. Proteomics of colorectal cancer liver metastasis: A quantitative focus on drug elimination and pharmacodynamics effects (10.1111/bcp.15098). *Br J Clin Pharmacol.* 2021.
63. USFDA. Drug Development and Drug Interactions | Table of Substrates, Inhibitors and Inducers. https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers.
64. FDA. Multidiscipline Review - Talazoparib. 2020.
65. Schwenger E, Reddy VP, Moorthy G, et al. Harnessing Meta-analysis to Refine an Oncology Patient Population for Physiology-Based Pharmacokinetic Modeling of Drugs. *Clin Pharmacol Ther.* 2018;103(2):271-280.
66. Hsieh EM, Hornik CP, Clark RH, et al. Medication use in the neonatal intensive care unit. *Am J Perinatol.* 2014;31(9):811-821.
67. Emoto C, Fukuda T, Johnson TN, Neuhoff S, Sadhasivam S, Vinks AA. Characterization of Contributing Factors to Variability in Morphine Clearance Through PBPK Modeling Implemented With OCT1 Transporter. *CPT Pharmacometrics Syst Pharmacol.* 2017;6(2):110-119.
68. Hahn D, Emoto C, Euteneuer JC, Mizuno T, Vinks AA, Fukuda T. Influence of OCT1 Ontogeny and Genetic Variation on Morphine Disposition in Critically Ill Neonates: Lessons From PBPK Modeling and Clinical Study. *Clin Pharmacol Ther.* 2019;105(3):761-768.
69. Li CY, Hosey-Cojocari C, Basit A, Unadkat JD, Leeder JS, Prasad B. Optimized Renal Transporter Quantification by Using Aquaporin 1 and Aquaporin 2 as Anatomical Markers: Application in Characterizing the Ontogeny of Renal Transporters and Its Correlation with Hepatic Transporters in Paired Human Samples. *AAPS J.* 2019;21(5):88.
70. Streekstra EJ, Russel FGM, van de Steeg E, de Wildt SN. Application of proteomics to understand maturation of drug metabolizing enzymes and transporters for the optimization of pediatric drug therapy. *Drug Discov Today Technol.* 2021;39:31-48.
71. Prasad B, Gaedigk A, Vrana M, et al. Ontogeny of Hepatic Drug Transporters as Quantified by LC-MS/MS Proteomics. *Clin Pharmacol Ther.* 2016;100(4):362-370.
72. Mooij MG, van de Steeg E, van Rosmalen J, et al. Proteomic Analysis of the Developmental Trajectory of Human Hepatic Membrane Transporter Proteins in the First Three Months of Life. *Drug Metab Dispos.* 2016;44(7):1005-1013.
73. Hedman M, Antikainen M, Holmberg C, et al. Pharmacokinetics and response to pravastatin in paediatric patients with familial hypercholesterolaemia and in paediatric cardiac transplant recipients in relation to polymorphisms of the SLCO1B1 and ABCB1 genes. *Br J Clin Pharmacol.* 2006;61(6):706-715.
74. Wagner JB, Abdel-Rahman S, Gaedigk R, et al. Impact of Genetic Variation on Pravastatin Systemic Exposure in Pediatric Hypercholesterolemia. *Clin Pharmacol Ther.* 2019;105(6):1501-1512.
75. Wagner JB, Abdel-Rahman S, Van Haandel L, et al. Impact of SLCO1B1 Genotype on Pediatric Simvastatin Acid Pharmacokinetics. *J Clin Pharmacol.* 2018;58(6):823-833.
76. Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M. SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. *Pharmacogenet Genomics.* 2006;16(12):873-879.
77. Wagner JB, Abdel-Rahman S, Gaedigk A, et al. Impact of SLCO1B1 Genetic Variation on Rosuvastatin Systemic Exposure in Pediatric Hypercholesterolemia. *Clin Transl Sci.* 2020;13(3):628-637.
78. Wood CE, Cousins R, Zhang D, Keller-Wood M. Ontogeny of expression of organic anion transporters 1 and 3 in ovine fetal and neonatal kidney. *Exp Biol Med (Maywood).* 2005;230(9):668-673.
79. Lam J, Baello S, Iqbal M, et al. The ontogeny of P-glycoprotein in the developing human blood-brain barrier: implication for opioid toxicity in neonates. *Pediatr Res.* 2015;78(4):417-421.
80. Verscheijden LFM, van Hattem AC, Pertijs J, et al. Developmental patterns in human blood-brain barrier and blood-cerebrospinal fluid barrier ABC drug transporter expression. *Histochem Cell Biol.* 2020;154(3):265-273.
81. Cristea S, Krekels EHJ, Rostami-Hodjegan A, Allegaert K, Knibbe CAJ. The Influence of Drug Properties and Ontogeny of Transporters on Pediatric Renal Clearance through Glomerular Filtration and Active Secretion: a Simulation-Based Study. *AAPS J.* 2020;22(4):87.
82. Tasnif Y, Morado J, Hebert MF. Pregnancy-related pharmacokinetic changes. *Clin Pharmacol Ther.* 2016;100(1):53-62.

83. Isoherranen N, Thummel KE. Drug metabolism and transport during pregnancy: how does drug disposition change during pregnancy and what are the mechanisms that cause such changes? *Drug Metab Dispos.* 2013;41(2):256-262.
84. Hebert MF, Easterling TR, Kirby B, et al. Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: a University of Washington specialized center of research study. *Clin Pharmacol Ther.* 2008;84(2):248-253.
85. Martin-Suarez A, Sanchez-Hernandez JG, Medina-Barajas F, et al. Pharmacokinetics and dosing requirements of digoxin in pregnant women treated for fetal supraventricular tachycardia. *Expert Rev Clin Pharmacol.* 2017;10(8):911-917.
86. Peng J, Ladumor MK, Unadkat JD. Prediction of Pregnancy-Induced Changes in Secretory and Total Renal Clearance of Drugs Transported by Organic Anion Transporters. *Drug Metab Dispos.* 2021;49(10):929-937.
87. Bergagnini-Kolev MC, Hebert MF, Easterling TR, Lin YS. Pregnancy Increases the Renal Secretion of N(1)-methylnicotinamide, an Endogenous Probe for Renal Cation Transporters, in Patients Prescribed Metformin. *Drug Metab Dispos.* 2017;45(3):325-329.
88. Eyal S, Easterling TR, Carr D, et al. Pharmacokinetics of metformin during pregnancy. *Drug Metab Dispos.* 2010;38(5):833-840.
89. Liao MZ, Gao C, Bhatt DK, Prasad B, Mao Q. Quantitative Proteomics Reveals Changes in Transporter Protein Abundance in Liver, Kidney and Brain of Mice by Pregnancy. *Drug Metab Lett.* 2018;12(2):145-152.
90. Rodrigues AD, van Dyk M, Sorich MJ, et al. Exploring the Use of Serum-Derived Small Extracellular Vesicles as Liquid Biopsy to Study the Induction of Hepatic Cytochromes P450 and Organic Anion Transporting Polypeptides. *Clin Pharmacol Ther.* 2021;110(1):248-258.
91. Anoshchenko O, Prasad B, Neradugomma NK, Wang J, Mao Q, Unadkat JD. Gestational Age-Dependent Abundance of Human Placental Transporters as Determined by Quantitative Targeted Proteomics. *Drug Metab Dispos.* 2020;48(9):735-741.
92. Kozlosky D, Barrett E, Aleksunes LM. R egulation of Placental Efflux Transporters During Pregnancy Complications. *Drug Metab Dispos.* 2022.
93. Freriksen JJM, Schalkwijk S, Colbers AP, et al. Assessment of Maternal and Fetal Dolutegravir Exposure by Integrating Ex Vivo Placental Perfusion Data and Physiologically-Based Pharmacokinetic Modeling. *Clin Pharmacol Ther.* 2020;107(6):1352-1361.
94. Ke AB, Greupink R, Abduljalil K. Drug Dosing in Pregnant Women: Challenges and Opportunities in Using Physiologically Based Pharmacokinetic Modeling and Simulations. *CPT Pharmacometrics Syst Pharmacol.* 2018;7(2):103-110.
95. Tan ML, Zhao P, Zhang L, et al. Use of Physiologically Based Pharmacokinetic Modeling to Evaluate the Effect of Chronic Kidney Disease on the Disposition of Hepatic CYP2C8 and OATP1B Drug Substrates. *Clin Pharmacol Ther.* 2019;105(3):719-729.
96. Follman KE, Morris ME. Prediction of the Effects of Renal Impairment on Clearance for Organic Cation Drugs that Undergo Renal Secretion: A Simulation-Based Study. *Drug Metab Dispos.* 2018;46(5):758-769.
97. Grimstein M, Yang Y, Zhang X, et al. Physiologically Based Pharmacokinetic Modeling in Regulatory Science: An Update From the U.S. Food and Drug Administration's Office of Clinical Pharmacology. *J Pharm Sci.* 2019;108(1):21-25.
98. Prevymis(R) (Letermovir) Clinical Pharmacology & Biopharmaceutics Review USFDA. 2017; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/209939Orig1s000,209940Orig1s000ClinPharmR.pdf.
99. Asciminib Multidiscipline Review USFDA. 2021; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2021/215358Orig1s000,Orig2s000MultidisciplineR.pdf.
100. Brouwer KLR, Evers R, Hayden E, et al. Regulation of Drug Transport Proteins - From Mechanisms to Clinical Impact; A White Paper on Behalf of the ITC. *Clin Pharmacol Ther.* 2022.
101. Scotcher D, Melillo N, Tadimalla S, et al. Physiologically Based Pharmacokinetic Modeling of Transporter-Mediated Hepatic Disposition of Imaging Biomarker Gadoxetate in Rats. *Mol Pharm.* 2021;18(8):2997-3009.
102. van der Made TK, Fedecostante M, Scotcher D, et al. Quantitative Translation of Microfluidic Transporter in Vitro Data to in Vivo Reveals Impaired Albumin-Facilitated Indoxyl Sulfate Secretion in Chronic Kidney Disease. *Mol Pharm.* 2019;16(11):4551-4562.

103. Achour B, Al-Majdoub ZM, Grybos-Gajniak A, et al. Liquid Biopsy Enables Quantification of the Abundance and Interindividual Variability of Hepatic Enzymes and Transporters. *Clin Pharmacol Ther.* 2021;109(1):222-232.
104. Achour B, Gosselin P, Terrier J, et al. Liquid Biopsy for Patient Characterization in Cardiovascular Disease: Verification against Markers of Cytochrome P450 and P-Glycoprotein Activities. *Clin Pharmacol Ther.* 2022.
105. Giacomini KM, Yee SW, Koleske ML, et al. New and emerging research on SLC and ABC transporters in drug discovery and development: Outlook from the International Transporter Consortium. *Clin Pharmacol Ther.* 2022.
106. Rodrigues D, Rowland A. From Endogenous Compounds as Biomarkers to Plasma-Derived Nanovesicles as Liquid Biopsy; Has the Golden Age of Translational Pharmacokinetics-Absorption, Distribution, Metabolism, Excretion-Drug-Drug Interaction Science Finally Arrived? *Clin Pharmacol Ther.* 2019;105(6):1407-1420.
107. Morgan RE, Campbell SE, Yu CY, Sponseller CA, Muster HA. Comparison of the safety, tolerability, and pharmacokinetic profile of a single oral dose of pitavastatin 4 mg in adult subjects with severe renal impairment not on hemodialysis versus healthy adult subjects. *J Cardiovasc Pharmacol.* 2012;60(1):42-48.
108. Stangier J, Rathgen K, Stahle H, Mazur D. Influence of renal impairment on the pharmacokinetics and pharmacodynamics of oral dabigatran etexilate: an open-label, parallel-group, single-centre study. *Clin Pharmacokinet.* 2010;49(4):259-268.
109. Tirona RG, Kassam Z, Strapp R, et al. Apixaban and Rosuvas--tatin Pharmacokinetics in Nonalcoholic Fatty Liver Disease. *Drug Metab Dispos.* 2018;46(5):485-492.
110. Sundelin EIO, Gormsen LC, Heeboll S, et al. Hepatic exposure of metformin in patients with non-alcoholic fatty liver disease. *Br J Clin Pharmacol.* 2019;85(8):1761-1770.
111. Gormsen LC, Sundelin EI, Jensen JB, et al. In Vivo Imaging of Human 11C-Metformin in Peripheral Organs: Dosimetry, Biodistribution, and Kinetic Analyses. *J Nucl Med.* 2016;57(12):1920-1926.
112. Sundelin E, Gormsen LC, Jensen JB, et al. Genetic Polymorphisms in Organic Cation Transporter 1 Attenuates Hepatic Metformin Exposure in Humans. *Clin Pharmacol Ther.* 2017;102(5):841-848.
113. Wagner JB, Leeder JS, Reed G, Zhang N, Galetin A. Utility of coproprophyrin I to predict statin exposure in children and adolescents. *Clin Pharmacol Ther.* 2021;109 Suppl 1:S88.
114. Ono H, Tanaka R, Suzuki Y, et al. Factors Influencing Plasma Coproporphyrin-I Concentration as Biomarker of OATP1B Activity in Patients With Rheumatoid Arthritis. *Clin Pharmacol Ther.* 2021;110(4):1096-1105.
115. Mori D, Ishida H, Mizuno T, et al. Alteration in the Plasma Concentrations of Endogenous Organic Anion-Transporting Polypeptide 1B Biomarkers in Patients with Non-Small Cell Lung Cancer Treated with Paclitaxel. *Drug Metab Dispos.* 2020;48(5):387-394.
116. Tsuboya A, Kubota Y, Ishida H, et al. Minimal contribution of the hepatic uptake transporter OATP1B1 to the inter-individual variability in SN-38 pharmacokinetics in cancer patients without severe renal failure. *Cancer Chemother Pharmacol.* 2021;88(3):543-553.
117. Cheung KWK, Yoshida K, Cheeti S, et al. GDC-0810 Pharmacokinetics and Transporter-Mediated Drug Interaction Evaluation with an Endogenous Biomarker in the First-in-Human, Dose Escalation Study. *Drug Metab Dispos.* 2019;47(9):966-973.
118. Wang L, Collins C, Kelly EJ, et al. Transporter Expression in Liver Tissue from Subjects with Alcoholic or Hepatitis C Cirrhosis Quantified by Targeted Quantitative Proteomics. *Drug Metab Dispos.* 2016;44(11):1752-1758.
119. El-Khateeb E, Achour B, Al-Majdoub ZM, Barber J, Rostami-Hodjegan A. Non-uniformity of Changes in Drug-Metabolizing Enzymes and Transporters in Liver Cirrhosis: Implications for Drug Dosage Adjustment. *Mol Pharm.* 2021;18(9):3563-3577.
120. Cheong EJY, Teo DWX, Chua DXY, Chan ECY. Systematic Development and Verification of a Physiologically Based Pharmacokinetic Model of Rivaroxaban. *Drug Metab Dispos.* 2019;47(11):1291-1306.
121. Moj D, Maas H, Schaeftlein A, Hanke N, Gomez-Mantilla JD, Lehr T. A Comprehensive Whole-Body Physiologically Based Pharmacokinetic Model of Dabigatran Etexilate, Dabigatran and Dabigatran Glucuronide in Healthy Adults and Renally Impaired Patients. *Clin Pharmacokinet.* 2019;58(12):1577-1593.
122. Fuhr LM, Hanke N, Meibohm B, Lehr T. Effective Removal of Dabigatran by Idarucizumab or Hemodialysis: A Physiologically Based Pharmacokinetic Modeling Analysis. *Clin Pharmacokinet.* 2020;59(6):809-825.

123. Ogawa SI, Shimizu M, Yamazaki H. Plasma concentrations of pemafibrate with co-administered drugs predicted by physiologically based pharmacokinetic modeling in virtual populations with renal/hepatic impairment. *Xenobiotica.* 2020;50(9):1023-1031.
124. Verscheijden LFM, Litjens CHC, Koenderink JB, et al. Physiologically based pharmacokinetic/pharmacodynamic model for the prediction of morphine brain disposition and analgesia in adults and children. *PLoS Comput Biol.* 2021;17(3):e1008786.
125. Cristea S, Krekels EHJ, Allegaert K, et al. Estimation of Ontogeny Functions for Renal Transporters Using a Combined Population Pharmacokinetic and Physiology-Based Pharmacokinetic Approach: Application to OAT1,3. *AAPS J.* 2021;23(3):65.

FIGURE LEGENDS:

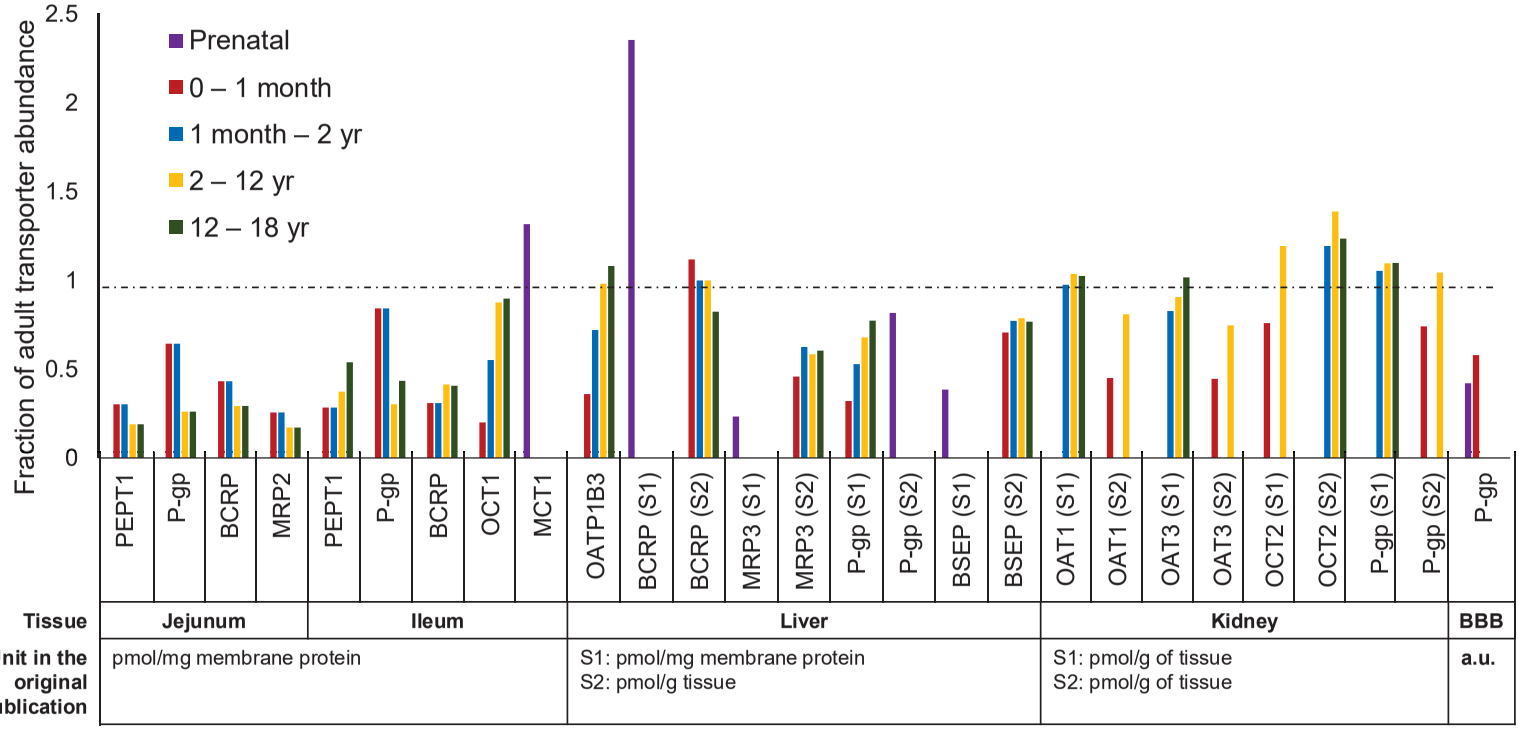
Figure 1: Transporter protein abundance in children represented as a fraction of adult levels across different age groups. Only clinically relevant transporters that have significant association with age are shown. Fractions have been calculated based on units in original publications as shown in the Figure. Expression data (mean ± sd) per individual transporter and age group are listed in **Table S3** and data references are provided in the Supplementary reading list.
a.u.: arbitrary unit; BCRP: breast cancer resistance protein; BSEP: bile salt export pump; MRP: multidrug resistance-associated protein; OAT: organic anion transporter, OATP: organic anion transporter polypeptide; OCT: organic cation transporter; PEPT1: proton-coupled oligopeptide transporter 1; P-gp: P-glycoprotein.

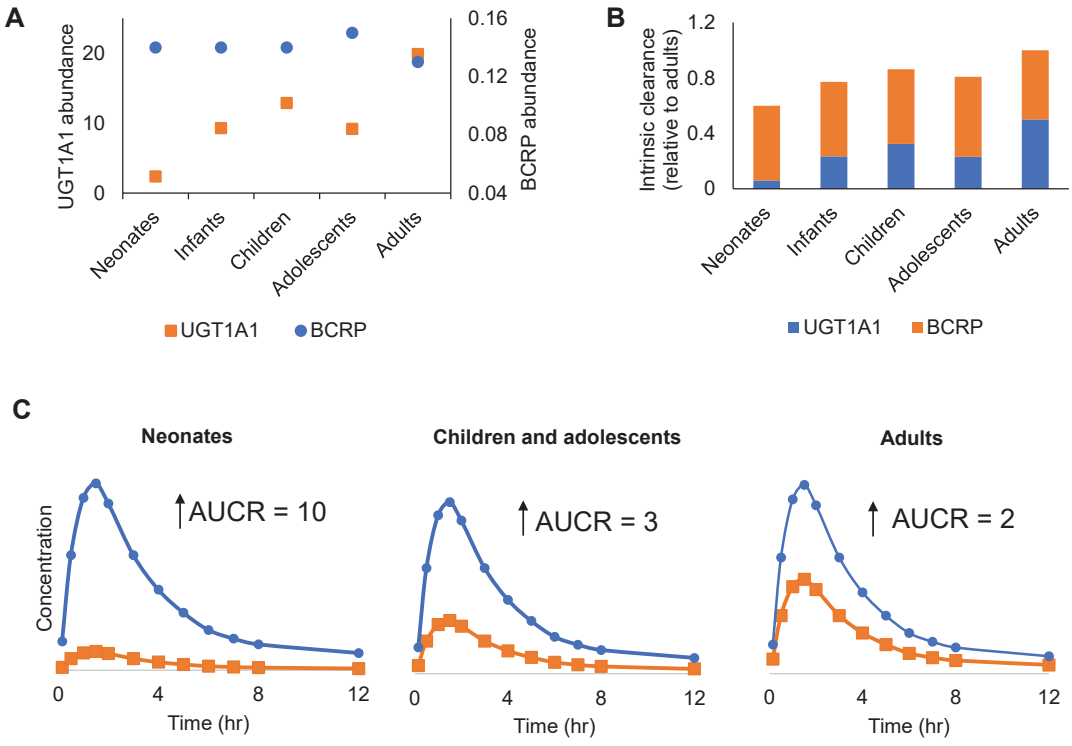
Figure 2: A hypothetical example demonstrating non-monotonic ontogeny profiles of UGT1A1 and BCRP (A), corresponding changes in the UGT1A1 and BCRP mediated hepatic intrinsic clearance (B) and pharmacokinetic profile in the liver in case of a complete inhibition of hepatic BCRP (C). For example, for a drug that is equally metabolized (by UGT1A1) and cleared by biliary excretion (by BCRP) in the liver, the contributions of UGT1A1 and BCRP in neonates are calculated ~10% and 90%, respectively. Thus, the effect of complete inhibition of the transporter activity by a DDI or genetic polymorphism would cause up to 10-fold increase in the hepatic concentration-time area under the curve (AUC) as compared to the maximum 2-fold change in adults. Blue and orange lines in C indicate hepatic concentration profiles with and without a complete inhibition of transporter activity, respectively.

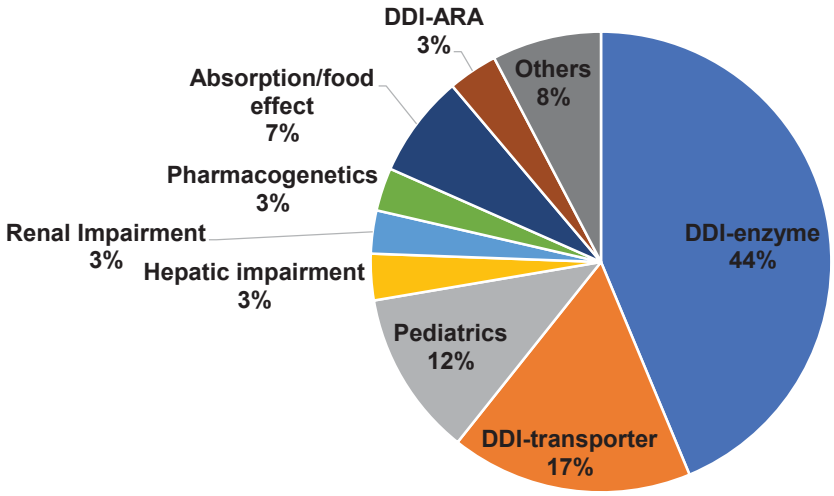
Figure 3: Analysis of PBPK submissions (2018-Dec 2021) to the Office of Clinical Pharmacology (OCP) at the U.S. FDA and distribution of PBPK application areas for this period. DDI: drug-drug interactions; ARA: acid-reducing agents

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).


Fraction of adult transporter abundance
2.5
2
1.5
1
0.5
0
Prenatal
0 – 1 month
1 month – 2 yr
2 – 12 yr
12 – 18 yr
PEPT1
P-gp
BCRP
MRP2
PEPT1
P-gp
BCRP
OCT1
MCT1
OATP1B3
BCRP (S1)
BCRP (S2)
MRP3 (S1)
MRP3 (S2)
P-gp (S1)
P-gp (S2)
BSEP (S1)
BSEP (S2)
OAT1 (S1)
OAT1 (S2)
OAT3 (S1)
OAT3 (S2)
OCT2 (S1)
OCT2 (S2)
P-gp (S1)
P-gp (S2)
P-gp
Tissue
Jejunum
Ileum
Liver
Kidney
BBB
Unit in the original publication
pmol/mg membrane protein
S1: pmol/mg membrane protein
S2: pmol/g tissue
S1: pmol/g of tissue
S2: pmol/g of tissue
a.u.


A
UGT1A1 abundance
20
10
0
0.16
0.12
0.08
0.04
BCRP abundance
Neonates
Infants
Children
Adolescents
Adults
UGT1A1
BCRP
B
Intrinsic clearance (relative to adults)
1.2
0.8
0.4
0
Neonates
Infants
Children
Adolescents
Adults
UGT1A1
BCRP
C
Neonates
Children and adolescents
Adults
Concentration
AUCR = 10
AUCR = 3
AUCR = 2
0
4
8
12
Time (hr)


DDI-ARA
3%
Others
8%
Absorption/food
effect
7%
Pharmacogenetics
3%
Renal Impairment
3%
Hepatic impairment
3%
DDI-enzyme
44%
Pediatrics
12%
DDI-transporter
17%

Supplementary Information for

Clinical implications of altered drug transporter abundance/function and PBPK modeling in specific populations: An ITC perspective

Xiaoyan Chu, Bhagwat Prasad, Sibylle Neuhoff, Kenta Yoshida, J. Steven Leeder, Dwaipayan Mukherjee, Kunal Taskar, Manthena Varma, Xinyuan Zhang, Xinning Yang and Aleksandra Galetin

Review of recently published (post-2017) clinical data from hepatic or renal impairment studies involving transporter substrate drugs

An initial data search was conducted using the University of Washington Drug Interaction Database (DIDB)[1]. 46 compounds could be identified that are substrates of one or more transporters and that have been approved after 2017 (compounds approved before that have been discussed in Guo et al.[2]) and have clinical studies in RI and HI patients. For 28 compounds out of these, transporter contributions were reported to be clinically relevant based on their clinical pharmacology reviews. Dual CYP and P-gp substrates were excluded from the analysis when they had significant metabolism (metabolism was > 50% of total elimination) and the compound was not a substrate of any transporters other than P-gp. This was due to the fact that there is a lack of proteomic data for P-gp transporter that describe the disease progression accurately in hepatic or renal impaired patients. In addition, CYP3A activities may be affected by hepatic impairment or certain degree of renal impairment (more likely in several renal impairment patients). Herein, we focus more on the involvement of transporters in these disease populations. The compounds which were not included in Table S1 (due to significant metabolism or being a substrate of only P-gp efflux) had less than 2-fold reported increases in hepatic or renal impairment individuals, except one compound (bazedoxifen), which had a reported exposure increase of more than 2-fold in hepatic impaired individuals.

The remaining dataset contained 10 compounds – atogepant, elagolix, velpatasvir, glecaprevir, pibrentasvir, ubrogepant, veliparib, grazoprevir, baricitinib, and letermovir (Table S1). Veliparib was an exception in that the drug has not been approved but it had published data in renally impaired patients and the compound is a substrate of multiple renal transporters (OCT2, MATE1/2K, P-gp) and has a renal clearance of 15 L/h[3] (73% of total clearance). Published clinical data for these 10 compounds in hepatic or renal impaired patients were summarized from the original publications. Table S1 also includes summarized details on the characteristics of the patients who were included in these clinical trials particularly their disease status and presence of other liver diseases. Compounds which had unbound concentrations measured in impaired patients were also reported in Table S1. In addition, Table S1 summarizes any available PBPK models for these compounds and these models were reviewed and only 6 of these 9 compounds had reported PBPK models which included their transporter-mediated disposition mechanisms. Out of these 6 compounds, only atogepant and letermovir had PBPK model-based predictions in organ impairment which were submitted or requested during regulatory approval.

For atogepant, PBPK model predictions were compared to clinically observed estimates (using population PK) in mild and moderate renal impairment and PBPK model were used to simulate drug exposures in severe renal impairment patients, and these were included in regulatory submission. However, the FDA did not consider these predictions to be reliable for dose adjustment in severe renal impairment[4]. There were 2 drugs which showed significant exposure changes in renal impairment - veliparib and baricitinib. Both were substrates of multiple renal transporters. Baricitinib is a substrate of P-gp, BCRP, OAT3, and MATE2K transporters and has 75% of its clearance attributed to renal elimination[5]. Veliparib is also a substrate of multiple renal transporters[6]. For both of these compounds there are reported PBPK models developed including renal transporters however it is not clear if the models were used to predict the impact of renal impairment.

Glecaprevir had a 2-fold and a 11-fold increase in AUC in moderate and severe hepatic impairment respectively which could be related to changes in OATP1B1/1B3, P-gp, or BCRP transporter expression in hepatic impairment (Table 2). Pibrentasvir showed a 5-fold increase in AUC in severe hepatic impairment and it is also a substrate of P-gp and BCRP efflux with no metabolic clearance. This might be related to changes in efflux transporter levels in severe hepatic impairment. Letermovir is an example where a clinically verified PBPK model was included in the drug product submission. The PBPK model for letermovir included saturable OATP1B1 uptake in the liver to describe the nonlinear PK[7]. Since the hepatic impairment study with letermovir was conducted at a dose of 60 mg, there was a concern that the impact of HI could be different at the clinically recommended dose of 480 mg, and the PBPK model was used to address the concern. Based on regulatory review, sensitivity analysis was conducted on the reduction of OATP transporter abundance at different dose levels and concluded that the change in letermovir PK is more sensitive to the change in OATP abundance at lower dose levels[7] (detailed discussion included in the main manuscript).

Table S1. Summary of clinical data (published post 2017) from hepatic or renal impairment studies involving drugs which are substrates of one or multiple transporters

Compound	Transporters involved (Other ADME properties)	HI/RI clinical studies (Demographics/study details)	HI/RI clinical data (% change from normal individuals in AUC/Cmax with variability in brackets)			Details/References
			Condition (N in brackets)	AUC**	Cmax	
Atogepant[8]	P-gp, BCRP, OATP1B1/1B3, OAT (~42% of dose metabolized primarily by CYP3A4, 5% renal, possible some biliary excretion)[4,8] 81% and 8% of radiolabeled dose recovered in feces and urine, with 42% and 5% of dose as unchanged drug in feces and urine; Single dose of rifampin increased AUC by 2.85-fold and Cmax by 2.23-fold.[4]	24 volunteers (8 in each cohort) with HI due to liver disease or cirrhosis (8 healthy volunteers as control); No HCV, HBV or HIV; no history of migraine; HI status by CP score[8] Renal impairment results from population PK analysis[4]	Mild HI* (8/32)	24.3 (-10.1 – 71.8)	9.0 (-27.3 – 63.3)	Unbound AUC in HI increased by 84%, 91%, 266%[8] PBPK model predictions were submitted to the FDA for RI but were not considered reliable to guide dose adjustment in severe RI at this point.[4]
			Moderate HI* (8/32)	15.0 (-16.8 – 59)	-11.8 (-41.2 – 32.1)	
			Severe HI* (8/32)	38.0 (-0.2 – 90.7)	-4.3 (-36.1 – 43.4)	
			Mild RI[b] (161)	1.7	-	
			Moderate RI[b] (13)	7.8	-	
Elagolix[9]	P-gp, OATP1B1 (~64% of dose metabolized –	Women (18-60 yr) in general good health except HI or RI; Not pregnant or	Mild HI* (6/22)	-20 (-52 – 33)	-18 (-50 – 34)	PBPK model: Chiney et al., 2020[11], Updated PBPK

	CYP3A4 (major), CYP2C8, CYP2D6, UGTs, 3% renal, possible some biliary excretion) 90% and <3% of radiolabeled dose recovered in feces and urine, with 26% of dose in feces as unchanged drug; Single dose of rifampin increased Cmax and AUC by 4.4- and 5.6-fold resp.[10]	breastfeeding; HI status by CP score; RI status by eGFR using Cockcroft-gault method and MDRD method – both resulted in the same classification[9]	Moderate HI*,[a] (6/22)	171 (63 – 353)	164 (61 – 334)	model in FDA review of elagolix (NDA213388)[12] Variability could not be reported in % however, no statistical significance compared to normal renal function. PBPK model not used for impairment predictions.
			Severe HI *,[a] (4/22)	570 (278 – 1088)	522 (257 – 981)	
			Moderate/ Severe RI (3/15)	-26	-35	
			ESRD (6/15)	-13	-19	
Velpatasvir[13-15,c]	P-gp, BCRP, OATP1B1/1B3 (~23% of dose metabolized – by CYP3A4, 2C8, 2B6, minimal renal elimination, some biliary excretion) 94% of radiolabeled dose recovered in feces with 77% as unchanged drug, and 0.4% of dose recovered in urine; Cyclosporine increased AUC by ~2 fold.[13]	HCV-infected cirrhotic patients with mild and moderate HI compared to HCV-infected noncirrhotic patients, dosed with sofosbuvir (pooled data from Phase 2/3 studies using popPK).[13] Non-HCV infected subjects (18 – 70 yr) with moderate and severe HI (velpatasvir alone); HI classification by CPT score;[15] non-HCV-infected subjects with severe RI (eGFR < 30 mL/min)	Mild HI (HCV) w/ sofosbuvir[b] (285)	-9.2 (-13.9 - -4.2)	-11 (-16.1 - -5.5)	PBPK model: Sane et al., 2020[16], Costales et al., 2021[17] (PBPK models published for the compound as a perpetrator only)
			Moderate HI (HCV) w/ sofosbuvir[b] (266)	-29.9 (-33.7 - -25.8)	-46.6 (-49.8 - -43.1)	
			Moderate HI (10/33)	-17 (-42.5 – 20)	-40.6 (-60.2 - -11.3)	
			Severe HI (10/33)	14 (-25.3 – 73)	-52.8 (-70.7 - -24)	

			Severe RI (10/19)	49.9 (17.0 – 92.1)	10.9 (-9.2 – 35.4)	
Glecaprevir [18,19]	P-gp, BCRP, OATP1B1/1B3 (~28% of dose metabolized - mostly by CYP3A4, minimal renal, some biliary excretion) 92% of radiolabeled dose recovered in feces with 22.6% as unchanged drug, and 0.66% urine. 41.7% of dose in feces was a metabolite formed by microflora in gut; 400 mg cyclosporine increased AUC 5-fold[18]	HCV-negative subjects (18- 65 yrs) with HI status by CP score. HI included but not limited to liver cirrhosis, hepatitis B infection, alcoholic liver disease, or previous HCV infection. Negative for hepatitis A.[18]	Mild HI w/ 120 mg pibrentasvir* (6/24)	33 (-51 – 258)	1 (-62 – 170)	PBPK model: Mukherjee et al.[19] (PBPK model not part of NDA and not used for organ impairment predictions.)
			Moderate HI w/ 120 mg pibrentasvir* (6/24)	100 (-24 – 425)	38 (-47 – 259)	
			Severe HI w/ 120 mg pibrentasvir* (6/24)	1010 (303 – 2975)	378 (75 – 1210)	
Pibrentasvir[18,1917,1817,1817,18]	P-gp, BCRP (not metabolized, some biliary elimination) 96.6% of radiolabeled drug recovered in feces with negligible	HCV-negative subjects (18 – 65 yrs) with HI status by CP score. HI included but not limited to liver cirrhosis, hepatitis B infection, alcoholic liver disease, or	Mild HI alone* (6/24)	51 (-27 – 213)	24 (-34 – 134)	PBPK model: Mukherjee et al.[20] (PBPK model not part of NDA and not used for organ impairment predictions.)
			Moderate HI alone* (6/24)	31 (-36 – 168)	14 (-38 – 112)	

	metabolism observed, no measurable radioactivity in urine; 400 mg cyclosporine increased AUC 1.9-fold[18]	previous HCV infection. Negative for hepatitis A.[19]	Severe HI alone* (6/24)	415 (152 – 950)	22 (-34 – 126)	
			Mild HI w/ 300 mg glecaprevir* (6/24)	-20 (-52 – 34)	-16 (-42 – 21)	
			Moderate HI w/ 300 mg glecaprevir* (6/24)	26 (-27 – 116)	26 (-15 – 86)	
			Severe HI w/ 300 mg glecaprevir* (6/24)	114 (28 – 258)	-41 (-59 - - 15)	
Veliparib[2]	P-gp, OCT2, MATE1, MATE2K (72% renal, 18% metabolized, mainly by CYP2D6)[5]	Patients with solid tumors that are metastatic or cannot be removed by surgery (18 – 60 yr) for moderate and severe HI; No HIV.	Mild & Moderate HI	HI did not appear to affect exposure		Preliminary published data indicates significant increase in exposure in RI (PK parameters not reported)[20] Population PK analysis found 65% increase in AUC in moderate RI[2] PBPK model: Mukherjee et al.[5]
			Moderate RI	NR	NR	
			Severe RI	NR	NR	

Ubrogepant[21]	P-gp, BCRP, weak substrate of OATP1B1/1B3, OAT1 (45% of dose metabolized, mostly by CYP3A46%, 6% renal, some biliary excretion) 83% and 9.5% of radiolabeled dose recovered in feces and urine, with 42% and 6% of dose as unchanged drug in feces and urine, respectively; Verapamil (P-gp/moderate CYPA inhibitor) caused a 3.5-fold increase in AUC; Ketoconazole (P-gp/strong CYP3A inhibitor) caused a 9.7-fold increase in AUC [21]	28 subjects in total with mild, moderate, and severe HI (and healthy subjects) without other co-morbidities.[21]	Mild HI (8/28)	6	4	Variability in PK parameters not reported. No published PBPK model available.
			Moderate HI (8/28)	52	25	
			Severe HI (4/28)	115	40	
Grazoprevir [21,22,c]	OATP1B1/1B3, P-gp (minimal renal elimination, ~20% of dose metabolized, mostly by CYP3A4, some biliary excretion)	25 subjects with mild, moderate or severe HI (and 25 healthy subjects) (18 – 65 yr) with no history of HCV infection. HI status by CP-score. Protein binding was evaluated.[22,23]	Mild HI (8/50)	66 (5 – 161)	37 (-17 – 127)	PBPK models: Matsumoto et al., 2019[23]; Costales et al., 2021[17] (grazoprevir modeled as a perpetrator only) % unbound grazoprevir in plasma = 1.7% (mild) vs 1.2%, 2.1% (moderate) vs
			Moderate HI (9/50)	382 (160 – 793)	498 (184 – 1157)	

	94% and 0.2% of radiolabeled dose recovered in feces and urine, with 75% of dose as unchanged drug in feces; 400 mg cyclosporine caused a 15-fold increase in AUC [22]	35 Japanese patients with cirrhosis were evaluated as part of another clinical study[22]	Severe HI (8/50)	1068 (510 – 2135)	1201 (500 – 2721)	1.7%, 1.9% (severe) vs 1.7% in their corresponding healthy controls[22]; Cirrhotic Japanese patients had 1.91-fold and 2.16-fold increase in steady-state Cmax and AUC compared to non-cirrhotic Japanese patients with HCV[25]
			Severe RI w/ elbasvir (8/16)	65 (9 – 149)	66 (-1 – 177)	
Baricitinib [5,24]	P-gp, BCRP, OAT3, MATE2K (6% of dose metabolized mainly by CYP3A4; primarily eliminated by renal) 75% and 20% of administered dose was eliminated in urine and feces, respectively. Baricitinib was excreted predominately as unchanged drug in urine (69%) and feces (15%); Probenecid increased baricitinib AUC ~2-fold.[26]	8 subjects with moderate HI, healthy in other respects, no HIV, HBV, HCV or other liver diseases.[27] 44 subjects in renal impairment study with 8 subjects with ESRD (post-dialysis) not shown here. RI status by MDRD method.[27]	Moderate HI (8/16)	18 (-5.5 - 49)	8 (-12.3 – 33)	PBPK model: Posada et al.[25] Not studied in severe HI; PBPK model exists but not used for impairment predictions.
			Mild RI (10/44)	41 (15 – 74)	16 (-8 – 45)	
			Moderate RI (10/44)	122 (81 – 173)	46 (17 – 83)	
			Severe RI (8/44)	305 (225 – 403)	40 (11 – 78)	
			ESRD (pre-dialysis) (8/44)	141 (-6 – 200)	-12 (-30 – 12)	
Letermovir[6,c]	P-gp, BSEP, OATP1B1/1B3 (23% of dose metabolized,	HI Study: 8 female subjects in each of moderate and severe HI	Moderate HI (8/33)*	59 (-2 – 157)	37 (-13 – 117)	PBPK model: Wang et al.[31]

	by CYP3A, 2D6, and UGT1A1/3, minimal renal, some biliary excretion) 93% and <2% of radiolabeled dose recovered in feces and urine, with 70% as unchanged drug in feces; Non-linear PK attributed to saturation of OATP1B1; Cyclosporine increased oral letermovir AUC 2-fold; PGx data supports OATP contribution[6]	with age (18 - 65 years) matched healthy controls. Subjects were healthy in all other respects and HI was classified according to CP-score.[29] RI study: 8 subjects in each group – healthy, moderate and severe RI, age (50 – 79 years), Caucasian and with no other comorbidities.[30]	Moderate HI* (unbound)	81 (3 – 220)	56 (-7 – 163)	Based on regulatory feedback, sensitivity analysis was conducted using PBPK model for effect of HI on OATP1B1 abundance.[6]
			Severe HI* (8/33)	282 (194 – 397)	134 (91 – 188)	
			Severe HI* (unbound)	436 (286 – 644)	229 (133 – 363)	
			Moderate RI* (8/24)	92 (43 – 158)	25 (-13 – 82)	
			Moderate RI* (unbound)	115 (59 – 191)	41 (-2 – 101)	
			Severe RI* (8/24)	42 (-17 – 143)	6 (-25 – 51)	
			Severe RI* (unbound)	81 (4 – 212)	35 (-4 – 90)	

*% change estimated from reported geometric mean ratios (GMRs)
**AUCinf reported in most cases except where only AUCt was reported
% change estimated from ratios of reported mean exposures
a: Statistically significant change compared to normal hepatic/renal function
b: Estimated based on population PK analysis of clinical data from Phase2/3 studies
c: Velpatasvir first approved as dual combo in 2016 with sofosbuvir and then as a triple combo product with voxilaprevir in 2017; Grazoprevir first approved in 2016

Table S2. Overview of HCV samples analyzed for transporter expression

Study	Control				Co-morbidities				Expression		Transporter
	metastatic	fibrosis	fatty	normal	only HCV	Cirrhosis	HCC	Tumor	mRNA	protein	(for simplicity only the protein name is stated)
El-Khateeb_2021[26]	X					X	X			X	NTCP, ASBT, MCT1, OATP1A2, OATP1B1, OATP1B3, OATP2B1, OCT1, OCT3, OAT2, OAT4 MDR1, BCRP, BSEP, MRP2, MRP3, MRP4, MRP6
Drozdzik_2020a[33]	X					X				X	NTCP, OATP1B1, OATP1B3, OATP2B1, OCT1, OCT3, OAT2 MDR1, BCRP, BSEP, MRP1, MRP2, MRP3, MRP4
Drozdzik_2020b[34]	X					X				X	MCT1
Billington_2017*[35]		X	X			X	X	X		X	NTCP, OATP1B1, OATP1B3, OATP2B1, OCT1, MATE1 MDR1, BSEP, MRP2, MRP3
Wang_2016*[27]		X	X			X				X	NTCP, OATP1B1, OATP1B3, OATP2B1, OCT1, MATE1 MDR1, BCRP, BSEP, MRP2, MRP3
Ogasawara_2010[28]	X				X	X			X	(MRP4)	OATP1B1, OATP1B3, OATP2B1, OCT1, OCTN2, OAT2, OAT7, MATE1, PEPT1 MDR1, MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, BCRP
Ros_2003[29]				X	X	X			X		MDR1, MDR3, BSEP, MRP1, MRP2, MRP3
Bonin_2002[30]				X		X			X		MDR1, MDR3, MRP1, MRP2, MRP3

* Same samples were analysed in Prasad_2016[31], Prasad_2014[32], Wang_2016[27] and Billington_2017[33].
\# Same samples were analysed in Drozdzik_2020a,b[34,35].

Transporter ontogeny data

The hepatic protein abundance data for OATP1B1 show no age-dependent changes in the protein expression except when the data are stratified using genotype information[31]. In particular, OATP1B1*14 and *15 also affect protein abundance[32] and potentially interfere with the interpretation of the ontogeny[31]. However, a clear trend of age-dependent increase between neonates and infants relative to the >1 year age group was observed when the analysis limited to donors with the OATP1B1 reference genotype, *1A/*1A. OATP1B3 protein abundance measured by LC-MS/MS proteomics data are conflicting. One study showed no association of age with OATP1B3 protein expression[36], whereas, the second study[31] showed age-dependent increase during the first year of life. OATP2B1 does not show any age-dependent changes in the protein abundance. Although NTCP levels do not show postnatal changes in the expression, fetal protein abundance is significantly lower than the adults[37]. Regarding the efflux transporters, mRNA and protein expression data of P-gp show lower abundance in fetal, neonatal, or infant tissues as compared to adults. However, one proteomics study showed no association of P-gp protein abundance with age. Similarly, a recent proteomic study suggests ~3-fold lower MRP2 abundance in fetal and newborn livers compared with adults[36], as opposed to other study which reported no change in MRP2[31]. MRP3 protein abundance was ~3-fold lower in fetal samples compared to adults in one study[36], and the levels in adolescents are lower than adults in another study[31]. While protein ontogeny data on MRP1, MRP4, and MRP6 are limited, hepatic MRP1 levels in livers from fetuses and neonates have been shown to be ~2-fold lower than those in adults.[38] Interestingly, while immunohistochemistry data detect BCRP in fetal liver at 5.5 weeks gestational age,[38] the mRNA expression is lower than in adults.[39] In contrast, the protein data appear to be comparable in fetuses when compared to children and adults.[31] Deeper statistical analysis of BCRP protein data suggest modest decrease in its hepatic abundance with age.[37] Lower hepatic BSEP activity is reported when measured by taurocholate biliary efflux in fetal hepatocytes than in adult hepatocytes, which is supported by LC-MS/MS proteomics data.[39]

Table S3. Age-dependent protein abundance data of drug transporters in human intestine, liver, kidney, and BBB. PTN = prenatal neonates, TN = full-term neonates.

Tissue	Transporter	Protein abundance (number of donors or detected/total donors)							Adult level reached	Reference
		Prenatal	Neonatal	Infancy	Childhood	Adolescence	Adulthood	Data expressed as (unit)		
			0-1 m	1 m – 2 yr	(2-12 yr	12-18 yr	>18 yr			
Jejunum	OATP2B1		37.1 ± 14.2 (8)		31.7 ± 14.7 (2)		43.2 ± 12.5 (8)	Mean ± SD (pmol/mg membrane protein)	>18 Yr	Kiss et al., 2021[40]
	MCT1		322.0 ± 308.9 (8)		156.3 ± 219.7 (1/2)		280 ± 69.9 (8)		0 – 1 m	Kiss et al., 2021[40]
	PEPT1		73.89 ± 47.6 (8)		46.4 ±38.7 (2)		243.9 ± 97 (8)		>18 Yr	Kiss et al., 2021[40]
	OCT1		6.7 ± 4.0 (6/8)		3.5 ± 3.3 (1/2)		7.2 ± 4.8 (6/8)		0 – 1 m	Kiss et al., 2021[40]
	P-gp		47.5 ± 37.8 (8)		19.3 ± 1.1 (2)		73.8 ± 29.7 (8)		>18 Yr	Kiss et al., 2021[40]
	BCRP		38.3 ± 30.4 (8)		26.0 ± 16.6 (2)		88.7 ± 37.0 (8)		>18 Yr	Kiss et al., 2021[40]
	MRP2		19.5 ± 8.8 (8)		13.0 ± 4.9 (2)		76.0 ± 39.6 (8)		>18 Yr	Kiss et al., 2021[40]
	MRP3		27.1 ± 20.6 (8)		8.7 ± 1.0 (2)		83.3 ± 37.7 (8)		>18 Yr	Kiss et al., 2021[40]
Ileum	OATP2B1		42.1 ± 18.1 (29)		40.7 ± 11.3 (6)	33.7 ± 16.6 (13)	27.3 ± 11.6		0 – 1 m	Kiss et al., 2021[40]
	MCT1		280.2 ± 374.5 (27/29)		108.7 ± 130.3 (4/6)	108.0 ± 171.4 (8/13)	295.5 ± 161.2 (8)		0 – 1 m	Kiss et al., 2021[40]
	PEPT1		49.1 ±29.5 (29)		64.7 ±43.6 (6)	93.2 ± 83.3 (13)	173.0 ±143.0 (8)		>18 Yr	Kiss et al., 2021[40]
	OCT1		5.7 ± 5.6 (16/29)		6.0 ± 8.0 (3/6)	2.5 ± 2.7 (3/13)	4.9 ± 4.5 (4/8)		0 – 1 m	Kiss et al., 2021[40]
	P-gp		106.8 ± 33.1 (29)		38.3 ± 17.4 (6)	55.1 ± 54.1 (13)	126.8 ± 99.6 (8)		>18 Yr	Kiss et al., 2021[40]

	BCRP		28.2 ± 17.7 (27/29)		37.7 ± 23.2 (5/6)	37.1 ± 33.9 (10/13)	91.1 ± 89.1 (8)		>18 Yr	Kiss et al., 2021[40]
	MRP2		20.6 ± 12.7 (27/29)		20.6 ± 13.7 (6)	19.9 ± 17.4 (13)	40.8 ± 32.2 (8)		>18 Yr	Kiss et al., 2021[40]
	MRP3		20.0 ± 17.5 (24/29)		22.9 ± 14.6 (6)	21.0 ± 17.5 (11/13)	52.6 ± 36.5 (8)		>18 Yr	Kiss et al., 2021[40]
Liver	OCT1*		0.94 ± 0.93 (4)	2.58 ± 1.11 (24)	4.1 ± 1.1 (27)	4.2 ± 1.23 (19)	4.68 ±1.91 (36)	Mean ± SD (pmol/mg membrane protein)	2-12 Yr	Prasad et al., 2016[31]
		66.7; 4.6-129.7 (36)	73.9; 42.7-139.7 (12)^{PTN} 45.9; 24.6-123.8 (10)^{TN}		86.1; 23.9-131.2 (4)		124.3; 60.1-181.4 (8)	Median; range (pmol/g tissue)		vanGroen et al., 2018[41]
	MCT1	11.2; 1.9-34.0 (36)	11.0; 5.8-27.9 (12)^{PTN} 9.2; 4.2-17.5 (10)^{TN}		8.9; 6.9-39.5 (4)		12.8; 5.7-20.6 (8)	Median; range (pmol/g tissue)		vanGroen et al., 2018[41]
	OATP1B1		2.71 ± 0.82 (4)	3.12 ± 1.59 (24)	3.28 ± 1.68 (27)	3.50 ± 1.49 (19)	4.13 ± 1.88 (36)	Mean ± SD (pmol/mg membrane protein)	2-12 Yr	Prasad et al., 2016[31]
		26.6; 7.1-101.7 (36)	15.0; 7.0-46.8 (12)^{PTN} 11.3; 4.9-16.5 (10)^{TN}		17.9; 8.2-23.2 (4)		17.7; 9.1-53.7 (8)	Median; range (pmol/g tissue)		vanGroen et al., 2018[41]
	OATP1B3*		0.58 ± 0.26 (4)	1.16 ± 0.77 (24)	1.58 ± 0.64 (27)	1.74 ± 0.67 (19)	1.61 ± 0.79 (36)	Mean ± SD (pmol/mg membrane protein)	2 – 12 Yr	Prasad et al., 2016[31]
		16.0; 2.3-50.9 (36)	16.7; 5.8-59.3 (12)^{PTN} 19.9; 11.1-51.1 (10)^{TN}		21.9, 15.1-38.9 (4)		16.0; 7.9-28.1 (8)	Median; range (pmol/g tissue)		vanGroen et al., 2018[41]

	OATP2B1	61.6; 13.8-123.8 (36)	75.5; 13.5-138.7 (12)[PTN] 51.9; 17.5-102.6 (10)[TN]		77.7 (60.8-79.1)		61.3 (34.5-77.8)	Median; range (pmol/g tissue)		
			1.17 ± 0.67 (4)	1.79 ± 0.64 (24)	1.44 ± 0.73 (27)	1.44 ± 0.72 (19)	1.67 ± 0.58 (36)	Mean ± SD (pmol/mg membrane protein)	2 – 12 Yr	Prasad et al., 2016[31]
	NTCP		0.67 ± 0.58 (4)	0.64 ± 0.15 (24)	0.73 ± 1.42 (27)	0.72 ± 0.56 (19)	2.16 ± 0.87 (36)		>18 Yr	
		2.3; 0.6-12.2 (36)	3.1; 0.9-15.3 (12)[PTN] 13.1; 1.5-18.0 (10)[TN]		17.7; 8.8-30.1 (4)		43.8; 10.2-81.6 (8)	pmol/g tissue		vanGroen et al., 2018[41]
	BCRP	3.9; 1.7-7.7 (36)	3.1; 0.9-7.3 (12)[PTN] 2.9; 1.6-6.8 (10)[TN]		3.7; 2.2-4.5 (4)		4.2; 1.6-6.5 (8)		0 – 1 m	
			0.19 ± 0.01 (3/4)	0.17 ± 0.07 (18/24)	0.17 ± 0.07 (21/27)	0.14 ± 0.04 (17/19)	0.17 ± 0.14	Mean ± SD (pmol/mg membrane protein)	0 – 1 m	Prasad et al., 2016[31]
	MRP2	8.0; 1.5-15.3 (36)	10.5; 6.5-23.9 (12)[PTN] 10.9; 3.4-20.2 (10)[TN]		8.9; 7.0-11.0 (4)		29.5; 19.6-39.6 (8)	Median; range (pmol/g tissue)		vanGroen et al., 2018[41]
	MRP3	1.8; 0.5-7.7 (36)	3.8; 1-11.3 (12)[PTN] 2.4; 0.9-13.7 (8)[TN]		6.2; 3.3-9.5 (4)		6.8; 4.2-9.9 (8)	Median; range (pmol/g tissue)		vanGroen et al., 2018[41]
			0.22 ± 0.23 (4)	0.30 ± 0.20 (24)	0.28 ± 0.19 (27)	0.29 ± 0.18 (19)	0.48 ± 0.15 (36)	Mean ± SD (pmol/mg membrane protein)	>18 Yr	
	OCTN2	6.3	4.4				3	pmol/g tissue	0 – 1 m	

	P-gp*		0.17 ± 0.10 (4)	0.28 ± 0.20 (24)	0.36 ± 0.18 (27)	0.41 ± 0.17 (19)	0.53 ± 0.22 (36)	Mean ± SD (pmol/mg membrane protein)	>18 Yr	Prasad et al., 2016[31]
		8.3; 0.04-24.9 (36)	9.2; 2.5-23.3 (12)^{PTN} 9.1; 1.5-20.7 (10)^{TN}		19.2; 10.6-26.7 (4)		20.5; 12.3-25.7 (8)	Median; range (pmol/g tissue)	0 – 1 m	vanGroen et al., 2018[41]
	BSEP	12.8; 1.3-45.2 (36)	25.7; 0.9-52.4 (12)^{PTN} 30.1; 9.8-47.7 (10)^{TN}		41.7; 20.4-59.7 (4)		60.1; 25.7-92.4 (8)		>18 Yr	vanGroen et al., 2018[41]
			1.49 ± 0.47 (4)	1.63 ± 0.59 (24)	1.66 ± 0.65 (27)	1.62 ± 0.46 (19)	2.11 ± 0.65 (36)	Mean ± SD (pmol/mg membrane protein)	>18 Yr	Prasad et al., 2016[31]
	MATE1		0.31 ± 0.01 (4)	0.37 ± 0.12 (24)	0.28 ± 0.16 (27)	0.25 ± 0.11 (19)	0.45 ± 0.15 (36)		>18 Yr	Prasad et al., 2016[31]
Kidney	OAT1			196.5 (2)	208.6 ± 59.1 (8)	206.5 ± 37.4 (12)	201.4 ± 61.9 (15)		0 – 1 m	Li et al., 2019[42]
			1.98 (100)		3.55 (38)		4.39 (27)		2- 12 Yr	Cheung et al., 2019[43]
	OAT3			121.7 (2)	133.5 ± 34.1 (8)	149.6 ± 33.5 (12)	147.2 ± 44.9 (15)		2- 12 Yr	Li et al., 2019[42]
			11.92 (100)		19.96 (38)		26.71 (27)			Cheung et al., 2019[43]
	OAT4			27.7 (2)	24.7 ± 11.1 (8)	25.9 ± 6.9 (12)	21.8 ± 6.6 (15)		0 – 1 m	Li et al., 2019[42]
			22.48 (100)		35.32 (38)		29.59 (27)	pmol/mg protein		Cheung et al., 2019[43]
				310.1 (2)	360.7 ± 79.7 (8)	321.0 ± 59.2 (12)	259.9 ± 96.2 (15)	Mean ± SD (pmol/gram of tissue)	0 – 1 m	Li et al., 2019[42]
	OCTN1			57.4 (2)	48.9 ± 11.4 (8)	57.4 ± 25.4 (12)	49.6 ± 10.2 (15)		0 – 1 m	
	OCTN2			26 (2)	29.6 ± 6.1 (8)	27.6 ± 5.4 (12)	25.6 ± 7.7 (15)		0 – 1 m	
	OATP4C1			43.2 (2)	45.1 ± 13.9 (8)	43.5 ± 9.1 (12)	39.3 ± 9.4 (15)		0 – 1 m	

	P-gp			89.2 (2)	92.8 ± 15.4 (8)	93.0 ± 16.3 (12)	84.7 ± 24.9 (15)		0 – 1 m	
			51.79 (100)		73.01 (38)		69.92 (27)	pmol/mg protein	0 – 1 m	Cheung et al., 2019[43]
	MRP2			69.6 (2)	63.1 ± 16.7 (8)	65.1 ± 17.1 (12)	55.9 ± 14.7 (15)	Mean ± SD (pmol/gram of tissue)	0 – 1 m	Li et al., 2019[42]
	MRP4									Li et al., 2019[42]
	BCRP		0.38 (100)		0.41 (38)		0.48 (27)	pmol/mg protein	0 – 1 m	Cheung et al., 2019[43]
	MATE1		16.36 (100)		18.00 (38)		19.56 (27)			Cheung et al., 2019[43]
				248.4 (2)	223.0 ± 48.1	213.2 ± 60.3 (12)	190.4 ± 58.8 (15)	Mean ± SD (pmol/gram of tissue)	0 – 1 m	Li et al., 2019[42]
	MATE2K		0.24 (100)		0.20 (38)		0.26 (27)	pmol/mg protein	0 – 1 m	Cheung et al., 2019[43]
BBB	P-gp	0.8 (12)	1.1 (8)				1.9 (8)	Relative (arbitrary unit)	>18 Yr	Lam et al., 2015[44]

*The estimated Age50 and Hill constant (h) for hepatic abundance of OCT1, OATP1B3, and P-gp are 0.47 and 0.92, 0.58 and 4.87, and 2.94 and 0.78, respectively.[31]

Table S4. Pharmacokinetic data for statins in pediatric population across different *SLCO1B1* groups

Drug	Age Range	Dose	AUC c.521TT (ng*h/mL)	AUC c.521TC (ng*h/mL)	AUC c.521CC (ng*h/mL)	AUC Ratio TC/TT	AUC Ratio CC/TT	References
Simvastatin	8-20 y (mean: 14 y)	10 mg (8-17 y); 20 mg (≥18 y)	1.9 ± 1.8	4.5 ± 2.5	12.1 ± 0.3	2.4	6.3	Wagner et al., 2018[45]
Pravastatin	8-20 y (mean: 14 y)	20 mg (8-13 y); 40 mg (≥14 y)	133.1 ± 113.4	225.4 ± 127.4	293.8 ± 16.1	1.7	2.2	Wagner et al., 2019[46]
Rosuvastatin	8-21 y (mean: 15 y)	10 mg	36.0 ± 18.5	49.2 ± 14.0	79.7 ± 18.2	1.4	2.2	Wagner et al., 2020[47]

Table S5: Meta-analysis of reported baseline coproporphyrins (CP) concentrations in healthy population

(a) Coproporphyrin I

Study references	Concentration [nM]		N	Weight (%)		Notes
	Mean	SD		(fixed)	(random)	
Lai Y 2016[48]	0.871	0.206	11	0.8	6.8	
Kunze 2018[49]	0.577	0.180	13	1.2	7.1	
Takehara I 2018[50]	0.700	0.067	8	5.5	7.5	
Shen H 2018[51]	0.715	0.149	16	2.2	7.3	
Mori D 2019[52]	0.563	0.034	10	26.8	7.6	CP-I levels from subjects with SLCO1B1*1b/*1b
Yee SW 2019[53]	0.600	0.100	8	2.4	7.3	CP-I levels from subjects with SLCO1B1 521TT
Mori D 2020[52]	0.833	0.034	8	21.1	7.6	
Tatosian DA 2021[54]	0.391	0.127	6	1.1	7.0	
Zhang Y 2020[55]	1.162	0.479	14	0.2	5.1	
Suzuki Y 2021[56]	0.687	0.183	103	9.4	7.5	CP-I levels from subjects with SLCO1B1*1b/*1b

Kalluri HV 2021[57]	0.808	0.097	10	3.2	7.4	
Feng S 2021[58]	0.287	0.067	17	11.5	7.5	
Suzuki Y 2021[59]	0.703	0.244	265	13.5	7.5	CP-I levels from subjects without SLCO1B1*15
Sane RS 2021[67]	0.846	0.213	15	1.0	7.0	
Fixed effect model	0.643 [0.632; 0.654]					
Random effect model	0.685 [0.587; 0.782]					

(b) Coproporphyrin III

Study References	Concentration [nM]		N	Weight (%)		Notes
	Mean	SD		(fixed)	(random)	
Lai Y 2016[48]	0.157	0.050	11	3.4	9.2	
Kunze 2018[49]	0.080	0.022	13	21.6	13.7	
Shen H 2018[51]	0.133	0.107	16	1.1	5.0	
Yee SW 2019[53]	0.110	0.020	8	15.6	13.2	CP-I levels from subjects with SLCO1B1 521TT
Tatosian DA 2021[54]	0.106	0.033	6	4.2	9.9	
Zhang Y 2020[55]	0.148	0.057	14	3.3	9.1	
Kalluri HV 2021[57]	0.106	0.024	10	13.2	13.0	
Feng S 2021[58]	0.086	0.024	17	22.9	13.8	
Sane RS 2021[60]	0.106	0.028	15	14.7	13.1	
Fixed effect model	0.100 [0.094; 0.105]					
Random effect model	0.110 [0.096; 0.124]					

Supplementary method for Meta-analysis of baseline coproporphyrin (CP) levels in healthy population

MEDLINE/PubMed was searched using the keywords “coproporphyrin OATP” with publication on or after 2016. Among 53 articles, we selected articles in which (1) concentrations or area-under-concentration time curve (AUC) in healthy or general population were reported and (2) mean or geometric mean and associated variability were reported in numeric values (i.e. excluded articles that only has graphical representation of data). For CP-I and CP-III, 14 and 9 articles were selected for the meta-analysis, respectively. When articles evaluated CP levels separately across different genotypes of SLCO1B1 subgroups, data from 521TT, *1b/*1b, or subjects without SLCO1B1*15 were used. Meta-analysis of the coproporphyrin levels was performed with both fixed and random effect models using meta package version 4.19-0 under R version 4.0.2.

References

1. University of Washington Drug Interaction Database (DIDB). https://www.druginteractionsolutions.org Accessed on: 03/2021.
2. Guo Y, Chu X, Parrott NJ, et al. Advancing Predictions of Tissue and Intracellular Drug Concentrations Using In Vitro, Imaging and Physiologically Based Pharmacokinetic Modeling Approaches. *Clin Pharmacol Ther.* 2018;104(5):865-889.
3. Stodtmann S, Nuthalapati S, Eckert D, et al. A Population Pharmacokinetic Meta-Analysis of Veliparib, a PARP Inhibitor, Across Phase 1/2/3 Trials in Cancer Patients. *J Clin Pharmacol.* 2021;61(9):1195-1205.
4. Qulipta(R) (Atogepant) Integrated Review USFDA(CDER). 2021; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2021/215206Orig1s000IntegratedR.pdf.
5. Olumiant(R) (Baricitinib) Clinical Pharmacology & Biopharmaceutics Review USFDA(CDER). 2016; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/207924Orig1s000ClinPharmR.pdf.
6. 2020 Annual Meeting American College of Clinical Pharmacology(R). *Clin Pharmacol Drug Dev.* 2020;9 Suppl 2:1-75.
7. Prevymis(R) (Letermovir) Clinical Pharmacology & Biopharmaceutics Review. 2017; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/209939Orig1s000,209940Orig1s000ClinPharmR.pdf.
8. Boinpally R, Jakate A, Butler M, Borbridge L, Periclou A. Single-Dose Pharmacokinetics and Safety of Atogepant in Adults With Hepatic Impairment: Results From an Open-Label, Phase 1 Trial. *Clin Pharmacol Drug Dev.* 2021;10(7):726-733.
9. Ng J, Duan WR, Marbury T, Schmidt JM, Klein CE. Elagolix Pharmacokinetic Profiles in Women With Renal or Hepatic Impairment. *Clin Pharmacol Drug Dev.* 2019;8(8):1053-1061.
10. Orilissa(R) (Elagolix) Multi Discipline Review. 2018; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210450Orig1s000MultiD.pdf.
11. Chiney MS, Ng J, Gibbs JP, Shebley M. Quantitative Assessment of Elagolix Enzyme-Transporter Interplay and Drug-Drug Interactions Using Physiologically Based Pharmacokinetic Modeling. *Clin Pharmacokinet.* 2020;59(5):617-627.
12. Oriahnn(R) (Elagolix) Multi Discipline Review. 2020; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/213388Orig1s000MultidisciplineR.pdf.
13. Epclusa(R) (Sofosbuvir/Velpatasvir) Clinical Pharmacology & Biopharmaceutics Review. 2016; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/208341Orig1s000ClinPharmR.pdf.
14. Vosevi(R) (Sofosbuvir/Velpatasvir/Voxilaprevir) Clinical Pharmacology & Biopharmaceutics Review. 2017; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/209195Orig1s000ClinPharmR.pdf.
15. Mogalian E, Brainard DM, Osinusi A, et al. Pharmacokinetics and Safety of Velpatasvir and Sofosbuvir/Velpatasvir in Subjects with Hepatic Impairment. *Clin Pharmacokinet.* 2018;57(11):1449-1457.
16. Sane R, Cheung KWK, Kovacs P, et al. Calibrating the In Vitro-In Vivo Correlation for OATP-Mediated Drug-Drug Interactions with Rosuvastatin Using Static and PBPK Models. *Drug Metab Dispos.* 2020;48(12):1264-1270.
17. Costales C, Lin J, Kimoto E, et al. Quantitative prediction of breast cancer resistant protein mediated drug-drug interactions using physiologically-based pharmacokinetic modeling. *CPT Pharmacometrics Syst Pharmacol.* 2021;10(9):1018-1031.
18. Mavyret(R) (Glecaprevir/Pibrentasvir) Clinical Pharmacology & Biopharmaceutics Review. 2017; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2017/209394Orig1s000ClinPharmR.pdf.
19. Kosloski MP, Wang H, Pugatch D, et al. Pharmacokinetics and safety of glecaprevir and pibrentasvir in HCV-negative subjects with hepatic impairment. *Eur J Clin Pharmacol.* 2019;75(2):217-226.
20. American Society for Clinical Pharmacology and Therapeutics. *Clinical Pharmacology & Therapeutics.* 2018;103(S1):S5-S97.
21. Zepatier(R) (Grazoprevir/Elbasvir) - Clinical Pharmacology & Biopharmaceutics Review. 2015; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/208261Orig1s000ClinPharmR.pdf.

22. Caro L, Wenning L, Guo Z, et al. Effect of Hepatic Impairment on the Pharmacokinetics of Grazoprevir, a Hepatitis C Virus Protease Inhibitor. *Antimicrob Agents Chemother.* 2017;61(12).
23. Matsumoto Y, Cabalu T, Sandhu P, et al. Application of Physiologically Based Pharmacokinetic Modeling to Predict Pharmacokinetics in Healthy Japanese Subjects. *Clin Pharmacol Ther.* 2019;105(4):1018-1030.
24. Co. EL. Olumiant(R) (baricitinib) US Prescribing Information. 2018; https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/207924s000lbl.pdf, 2022.
25. Posada MM, Cannady EA, Payne CD, et al. Prediction of Transporter-Mediated Drug-Drug Interactions for Baricitinib. *Clin Transl Sci.* 2017;10(6):509-519.
26. El-Khateeb E, Achour B, Al-Majdoub ZM, Barber J, Rostami-Hodjegan A. Non-uniformity of Changes in Drug-Metabolizing Enzymes and Transporters in Liver Cirrhosis: Implications for Drug Dosage Adjustment. *Mol Pharm.* 2021;18(9):3563-3577.
27. Wang L, Collins C, Kelly EJ, et al. Transporter Expression in Liver Tissue from Subjects with Alcoholic or Hepatitis C Cirrhosis Quantified by Targeted Quantitative Proteomics. *Drug Metab Dispos.* 2016;44(11):1752-1758.
28. Ogasawara K, Terada T, Katsura T, et al. Hepatitis C virus-related cirrhosis is a major determinant of the expression levels of hepatic drug transporters. *Drug Metab Pharmacokinet.* 2010;25(2):190-199.
29. Ros JE, Libbrecht L, Geuken M, Jansen PL, Roskams TA. High expression of MDR1, MRP1, and MRP3 in the hepatic progenitor cell compartment and hepatocytes in severe human liver disease. *J Pathol.* 2003;200(5):553-560.
30. Bonin S, Pascolo L, Croce LS, Stanta G, Tiribelli C. Gene expression of ABC proteins in hepatocellular carcinoma, perineoplastic tissue, and liver diseases. *Mol Med.* 2002;8(6):318-325.
31. Prasad B, Gaedigk A, Vrana M, et al. Ontogeny of Hepatic Drug Transporters as Quantified by LC-MS/MS Proteomics. *Clin Pharmacol Ther.* 2016;100(4):362-370.
32. Prasad B, Evers R, Gupta A, et al. 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.* 2014;42(1):78-88.
33. Billington S, Ray AS, Salphati L, et al. Transporter Expression in Noncancerous and Cancerous Liver Tissue from Donors with Hepatocellular Carcinoma and Chronic Hepatitis C Infection Quantified by LC-MS/MS Proteomics. *Drug Metab Dispos.* 2018;46(2):189-196.
34. Drozdzik M, Szelag-Pieniek S, Post M, et al. Protein Abundance of Hepatic Drug Transporters in Patients With Different Forms of Liver Damage. *Clin Pharmacol Ther.* 2020;107(5):1138-1148.
35. Drozdzik M, Szelag-Pieniek S, Grzegolkowska J, et al. Monocarboxylate Transporter 1 (MCT1) in Liver Pathology. *Int J Mol Sci.* 2020;21(5).
36. Mooij MG, van de Steeg E, van Rosmalen J, et al. Proteomic Analysis of the Developmental Trajectory of Human Hepatic Membrane Transporter Proteins in the First Three Months of Life. *Drug Metab Dispos.* 2016;44(7):1005-1013.
37. van Groen BD, Nicolai J, Kuik AC, et al. Ontogeny of Hepatic Transporters and Drug-Metabolizing Enzymes in Humans and in Nonclinical Species. *Pharmacol Rev.* 2021;73(2):597-678.
38. Konieczna A, Erdosova B, Lichnovska R, Jandl M, Cizkova K, Ehrmann J. Differential expression of ABC transporters (MDR1, MRP1, BCRP) in developing human embryos. *J Mol Histol.* 2011;42(6):567-574.
39. Sharma S, Ellis EC, Gramignoli R, et al. Hepatobiliary disposition of 17-OHPC and taurocholate in fetal human hepatocytes: a comparison with adult human hepatocytes. *Drug Metab Dispos.* 2013;41(2):296-304.
40. Kiss M, Mbasu R, Nicolai J, et al. Ontogeny of Small Intestinal Drug Transporters and Metabolizing Enzymes Based on Targeted Quantitative Proteomics. *Drug Metab Dispos.* 2021;49(12):1038-1046.
41. van Groen BD, van de Steeg E, Mooij MG, et al. Proteomics of human liver membrane transporters: a focus on fetuses and newborn infants. *Eur J Pharm Sci.* 2018;124:217-227.
42. Li CY, Hosey-Cojocari C, Basit A, Unadkat JD, Leeder JS, Prasad B. Optimized Renal Transporter Quantification by Using Aquaporin 1 and Aquaporin 2 as Anatomical Markers: Application in Characterizing the Ontogeny of Renal Transporters and Its Correlation with Hepatic Transporters in Paired Human Samples. *AAPS J.* 2019;21(5):88.

43. Cheung KWK, van Groen BD, Spaans E, et al. A Comprehensive Analysis of Ontogeny of Renal Drug Transporters: mRNA Analyses, Quantitative Proteomics, and Localization. *Clin Pharmacol Ther.* 2019;106(5):1083-1092.
44. Lam J, Baello S, Iqbal M, et al. The ontogeny of P-glycoprotein in the developing human blood-brain barrier: implication for opioid toxicity in neonates. *Pediatr Res.* 2015;78(4):417-421.
45. Wagner JB, Abdel-Rahman S, Van Haandel L, et al. Impact of SLCO1B1 Genotype on Pediatric Simvastatin Acid Pharmacokinetics. *J Clin Pharmacol.* 2018;58(6):823-833.
46. Wagner JB, Abdel-Rahman S, Gaedigk R, et al. Impact of Genetic Variation on Pravastatin Systemic Exposure in Pediatric Hypercholesterolemia. *Clin Pharmacol Ther.* 2019;105(6):1501-1512.
47. Wagner JB, Abdel-Rahman S, Gaedigk A, et al. Impact of SLCO1B1 Genetic Variation on Rosuvastatin Systemic Exposure in Pediatric Hypercholesterolemia. *Clin Transl Sci.* 2020;13(3):628-637.
48. Lai Y, Mandlekar S, Shen H, et al. Coproporphyrins in Plasma and Urine Can Be Appropriate Clinical Biomarkers to Recapitulate Drug-Drug Interactions Mediated by Organic Anion Transporting Polypeptide Inhibition. *J Pharmacol Exp Ther.* 2016;358(3):397-404.
49. Kunze A, Ediage EN, Dillen L, Monshouwer M, Snoeys J. Clinical Investigation of Coproporphyrins as Sensitive Biomarkers to Predict Mild to Strong OATP1B-Mediated Drug-Drug Interactions. *Clin Pharmacokinet.* 2018;57(12):1559-1570.
50. Takehara I, Yoshikado T, Ishigame K, et al. Comparative Study of the Dose-Dependence of OATP1B Inhibition by Rifampicin Using Probe Drugs and Endogenous Substrates in Healthy Volunteers. *Pharm Res.* 2018;35(7):138.
51. Shen H, Christopher L, Lai Y, et al. Further Studies to Support the Use of Coproporphyrin I and III as Novel Clinical Biomarkers for Evaluating the Potential for Organic Anion Transporting Polypeptide 1B1 and OATP1B3 Inhibition. *Drug Metab Dispos.* 2018;46(8):1075-1082.
52. Mori D, Kashihara Y, Yoshikado T, et al. Effect of OATP1B1 genotypes on plasma concentrations of endogenous OATP1B1 substrates and drugs, and their association in healthy volunteers. *Drug Metab Pharmacokinet.* 2019;34(1):78-86.
53. Yee SW, Giacomini MM, Shen H, et al. Organic Anion Transporter Polypeptide 1B1 Polymorphism Modulates the Extent of Drug-Drug Interaction and Associated Biomarker Levels in Healthy Volunteers. *Clin Transl Sci.* 2019;12(4):388-399.
54. Tatosian DA, Yee KL, Zhang Z, et al. A Microdose Cocktail to Evaluate Drug Interactions in Patients with Renal Impairment. *Clin Pharmacol Ther.* 2021;109(2):403-415.
55. Zhang Y, Holenarsipur VK, Kandoussi H, et al. Detection of Weak Organic Anion-Transporting Polypeptide 1B Inhibition by Probenecid with Plasma-Based Coproporphyrin in Humans. *Drug Metab Dispos.* 2020;48(10):841-848.
56. Suzuki Y, Sasamoto Y, Koyama T, et al. Substantially Increased Plasma Coproporphyrin-I Concentrations Associated With OATP1B1*15 Allele in Japanese General Population. *Clin Transl Sci.* 2021;14(1):382-388.
57. Kalluri HV, Kikuchi R, Coppola S, et al. Coproporphyrin I Can Serve as an Endogenous Biomarker for OATP1B1 Inhibition: Assessment Using a Glecaprevir/Pibrentasvir Clinical Study. *Clin Transl Sci.* 2021;14(1):373-381.
58. Feng S, Bo Q, Coleman HA, et al. Further Evaluation of Coproporphyrins as Clinical Endogenous Markers for OATP1B. *J Clin Pharmacol.* 2021;61(8):1027-1034.
59. Suzuki Y, Sasamoto Y, Koyama T, et al. Relationship of hemoglobin level and plasma coproporphyrin-I concentrations as an endogenous probe for phenotyping OATP1B. *Clin Transl Sci.* 2021;14(4):1403-1411.
60. Sane RS, Cheung KWK, Cho E, et al. Evaluation of Ipatasertib Interactions with Itraconazole and Coproporphyrin I and III in a Single Drug Interaction Study in Healthy Subjects. *J Pharmacol Exp Ther.* 2021;378(2):87-95.

Supplementary Reading List

Organ Impairment

Tanaka K, Xu W, Zhou F, You G. Role of glycosylation in the organic anion transporter OAT1. *J Biol Chem*. 2004;279(15):14961-14966.

Canet MJ, Merrell MD, Hardwick RN, et al. Altered regulation of hepatic efflux transporters disrupts acetaminophen disposition in pediatric nonalcoholic steatohepatitis. *Drug Metab Dispos*. 2015;43(6):829-835.

Vildhede A, Kimoto E, Pelis RM, Rodrigues AD, Varma MVS. Quantitative Proteomics and Mechanistic Modeling of Transporter-Mediated Disposition in Nonalcoholic Fatty Liver Disease. *Clin Pharmacol Ther*. 2020;107(5):1128-1137.

Pelis RM, Zhang X, Dangprapai Y, Wright SH. Cysteine accessibility in the hydrophilic cleft of human organic cation transporter 2. *J Biol Chem*. 2006;281(46):35272-35280.

Puri P, Daita K, Joyce A, et al. The presence and severity of nonalcoholic steatohepatitis is associated with specific changes in circulating bile acids. *Hepatology*. 2018;67(2):534-548.

Jansen J, Jankowski J, Gajjala PR, Wetzels JFM, Masereeuw R. Disposition and clinical implications of protein-bound uremic toxins. *Clin Sci* (Lond). 2017;131(14):1631-1647.

Nigam SK, Bush KT. Uraemic syndrome of chronic kidney disease: altered remote sensing and signalling. *Nat Rev Nephrol*. 2019;15(5):301-316.

Ferslew BC, Xie G, Johnston CK, et al. Altered Bile Acid Metabolome in Patients with Nonalcoholic Steatohepatitis. *Dig Dis Sci*. 2015;60(11):3318-3328.

Kalhan SC, Guo L, Edmison J, et al. Plasma metabolomic profile in nonalcoholic fatty liver disease. *Metabolism*. 2011;60(3):404-413.

Danin PE, Anty R, Patouraux S, et al. Non-invasive Evaluation of NAFLD with Indocyanine Green Clearance Test: a Preliminary Study in Morbidly Obese Patients Undergoing Bariatric Surgery. *Obes Surg*. 2018;28(3):735-742.

Pediatric populations

Laughon MM, Avant D, Tripathi N, et al. Drug labeling and exposure in neonates. *JAMA Pediatr*. 2014;168(2):130-136.

Hsieh EM, Hornik CP, Clark RH, et al. Medication use in the neonatal intensive care unit. *Am J Perinatol*. 2014;31(9):811-821.

Hedman M, Neuvonen PJ, Neuvonen M, Antikainen M. Pharmacokinetics and pharmacodynamics of pravastatin in children with familial hypercholesterolemia. *Clin Pharmacol Ther*. 2003;74(2):178-185.

Hedman M, Neuvonen PJ, Neuvonen M, Holmberg C, Antikainen M. Pharmacokinetics and pharmacodynamics of pravastatin in pediatric and adolescent cardiac transplant recipients on a regimen of triple immunosuppression. *Clin Pharmacol Ther*. 2004;75(1):101-109.

Hepatitis C Infection

Meier A, Mehrle S, Weiss TS, Mier W, Urban S. Myristoylated PreS1-domain of the hepatitis B virus L-protein mediates specific binding to differentiated hepatocytes. *Hepatology.* 2013;58(1):31-42.

Yan H, Zhong G, Xu G, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. *Elife.* 2012;1:e00049.

Wang L, Collins C, Kelly EJ, Chu X, et al., Transporter Expression in Liver Tissue from Subjects with Alcoholic or Hepatitis C Cirrhosis Quantified by Targeted Quantitative Proteomics, *Drug Metabolism and Disposition*, 2016, 44 (11) 1752-1758

Bonin S. Pascolo L, Croce LS, Stanta G, Tiribelli, C, Gene expression of ABC proteins in hepatocellular carcinoma, perineoplastic tissue, and liver diseases*, Molecular Medicine*, 2012, 8: 318-325

Ros JE, Libbrecht L, Geuken M, Jansen PLM, Roskams TAD, High expression of MDR1, MRP1, and MRP3 in the hepatic progenitor cell compartment and hepatocytes in severe human liver disease, *J Pathol*, 2005, 200(5):553-560

Snoeys J, Beumont M, Monshouwer M, Ouwerkerk-Mahadevan S, Mechanistic understanding of the nonlinear pharmacokinetics and intersubject variability of simeprevir: A PBPK-guided drug development approach, *Clin Pharmacol Ther*. 2016 Feb;99(2):224-34

Snoeys J, Beumont M, Monshouwer M, Ouwerkerk-Mahadevan S, Elucidating the Plasma and Liver Pharmacokinetics of Simeprevir in Special Populations Using Physiologically Based Pharmacokinetic Modelling, *Clin Pharmacol Ther*. 2017 Jul;56(7):781-792

Costales C, Lin J, Kimoto E, et al. Quantitative prediction of breast cancer resistant protein mediated drug-drug interactions using physiologically-based pharmacokinetic modeling. *CPT Pharmacometrics Syst Pharmacol.* 2021;10(9):1018-1031.

Shebley M, Fu W, Badri P, Bow DAJ, Fischer V, Physiologically Based Pharmacokinetic Modeling Suggests Limited Drug–Drug Interaction Between Clopidogrel and Dasabuvir, *Clin Pharmacol Ther.* 2017; 102(4): 679–687.

Reddy MB, Bolger MB, Fraczkiewicz G, Frari LD, et al., PBPK Modeling as a Tool for Predicting and Understanding Intestinal Metabolism of Uridine 5′-Diphospho-glucuronosyltransferase Substrates, *Pharmaceutics*. 2021; 13(9): 1325.

Kimoto E, Vourvahis M, Scialis RJ, Eng H, Rodrigues D, Varma MVS, Mechanistic Evaluation of the Complex Drug-Drug Interactions of Maraviroc: Contribution of Cytochrome P450 3A, P-Glycoprotein and Organic Anion Transporting Polypeptide 1B1, *Drug Metab Dispos* 2019, 47 (5) 493-503

Sane R, Cheung KWK, Kovacs P, Farasyn T, et al., Calibrating the In Vitro–In Vivo Correlation for OATP-Mediated Drug-Drug Interactions with Rosuvastatin Using Static and PBPK Models, *Drug Metab Dispos*, 2020, 48 (12) 1264-1270;

Literature sources for proteomic data shown in Figure 1

Kiss M, Mbasu R, Nicolai J, et al. Ontogeny of Small Intestinal Drug Transporters and Metabolizing Enzymes Based on Targeted Quantitative Proteomics. *Drug Metab Dispos*. 2021;49(12):1038-1046. (Protein abundances for jejunum and ileum)

Prasad B, Gaedigk A, Vrana M, et al. Ontogeny of Hepatic Drug Transporters as Quantified by LC-MS/MS Proteomics. Clin Pharmacol Ther. 2016;100(4):362-370. (Protein abundances in the liver)

Mooij MG, van de Steeg E, van Rosmalen J, et al. Proteomic Analysis of the Developmental Trajectory of Human Hepatic Membrane Transporter Proteins in the First Three Months of Life. *Drug Metab Dispos*. 2016;44(7):1005-1013. (Protein abundances in the liver)

Cheung KWK, van Groen BD, Spaans E, et al. A Comprehensive Analysis of Ontogeny of Renal Drug Transporters: mRNA Analyses, Quantitative Proteomics, and Localization. *Clin Pharmacol Ther*. 2019;106(5):1083-1092. (Protein abundances in the kidney)

Li CY, Hosey-Cojocari C, Basit A, Unadkat JD, Leeder JS, Prasad B. Optimized Renal Transporter Quantification by Using Aquaporin 1 and Aquaporin 2 as Anatomical Markers: Application in Characterizing the Ontogeny of Renal Transporters and Its Correlation with Hepatic Transporters in Paired Human Samples. *AAPS J*. 2019;21(5):88. (Protein abundances in the kidney)

Lam J, Baello S, Iqbal M, et al. The ontogeny of P-glycoprotein in the developing human blood-brain barrier: implication for opioid toxicity in neonates. *Pediatr Res*. 2015;78(4):417-421. (Protein abundances in the blood-brain barrier)