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Research Article

Estimating *In Vivo* Fractional Contribution of OATP1B1 to Human Hepatic Active Uptake by Mechanistically Modeling Pharmacogenetic Data

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Abstract. A reasonable estimate on the fractional contribution of transporters to total hepatic active uptake (F_T) is a critical factor in understanding and predicting human clearance, drug-drug interaction, and pharmacokinetic variability for hepatic transporter substrates. F_T values for organic-anion-transporting polypeptide (OATP) 1B1 have been previously determined using *in vitro* assays. However, to date, none of the published *in vitro* F_T values has been validated against or compared with *in vivo* F_T values due to the lack of clinical data from selective substrates or inhibitors. The possible transporter-dependent *in vitro* to *in vivo* scaling further weakens the predictive power of these *in vitro*–determined F_T values. In facing this challenge, a method is developed in this study to estimate *in vivo* OATP1B1 F_T values by mechanistically modeling genotyped clinical pharmacokinetic data. The method is based on the hypothesis that observed change in hepatic active uptake clearance due to OATP1B1 polymorphism depends on two factors: (1) the contribution of OATP1B1 to the hepatic active uptake clearance and (2) the change of OATP1B1-mediated intrinsic uptake activity by the polymorphism. Conversely, if the changes caused by genetic variations in hepatic active uptake clearance and in OATP1B1-mediated clearance are known, then the OATP1B1 contribution to the hepatic active uptake clearance can be calculated. This is the first time that *in vivo* hepatic transporter F_T values and a method to estimate these values are reported. Both F_T values and the estimation method will facilitate future understanding and prediction on the transporter-mediated drug disposition.

KEY WORDS: OATP1B1; fractional contribution; hepatic transporter; modeling and simulation; pharmacogenetics.

INTRODUCTION

The accurate predictions of human pharmacokinetics, drug-drug interactions (DDI), and variability due to genetic mutations are important components in drug discovery and development, as the pharmacology and toxicology effects are ultimately driven by the exposure. Over the past decade, significant progress has been made to predict metabolic clearance mediated by hepatic cytochrome P450 (CYP). The advance, in conjunction with high-throughput screening assays and early metabolite identification, has enabled drug discovery teams to successfully increase the metabolic stability and avoid potential CYP-mediated DDI risk or pharmacokinetic variability before the new compounds enter clinical trials.

However, as the medicinal chemistry in the modern drug discovery being more and more successful in reducing CYP-mediated clearance, it has also led to an increased prevalence of hepatic transporter–mediated clearance (1). Although several *in vitro* assay systems have been developed so far to understand hepatic uptake transporter activity, translation from *in vitro* data to *in vivo* prediction is still challenging. For example, transporter uptake activity is usually different between *in vitro* and *in vivo* systems for unidentified reasons, so empirical scaling factors are likely required to extrapolate rates derived from *in vitro* assays to *in vivo* rates (2). Li and others have previously developed a method to estimate the unified scaling factors by simultaneously fitting training data of multiple compounds (3). However, the training compounds included in this study are mostly major organic-anion-transporting polypeptide (OATP) 1B1 and 1B3 substrates. Hence, it is unclear if the same empirical factors can be shared with the substrates of other hepatic transporters (e.g., sodium-taurocholate cotransporting polypeptide (NTCP) or organic anion transporters (OATs)).

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Since different transporters may have different degrees of disconnection between *in vitro* and *in vivo*, to accurately predict clearance, DDI, and variability due to genetic variation of transporters, a reasonable estimate on the fractional contribution of transporters to total hepatic active uptake (F_T) is necessary. F_T values can split the hepatic uptake clearance into processes mediated through individual transporters. Then, the uptake and its inhibition can be analyzed for individual transporters using transporter-specific scaling factors and inhibition potency, as described previously (4,5). Similar strategies have been used in predicting CYP-mediated DDI, where the fractional contribution of enzyme to total hepatic metabolism (F_M) is estimated from *in vitro* data (6). A few *in vitro* methods have been developed to understand F_T (7–14). Compared with *in vitro* F_M values, it is less convincing that *in vitro* F_T values are good surrogates of *in vivo* values, because of the possibly transporter-dependent *in vitro* to *in vivo* discrepancy. For example, assuming that transporters A and B contribute 50% and 50% of the *in vivo* total hepatic active uptake, if transporter A is less active *in vitro* than *in vivo*, but transporter B has similar *in vitro* and *in vivo* activities, then the measured *in vitro* F_T values will be less than 50% for A and over 50% for B. *In vitro* F_T values will be 50% for both, only if *in vitro* to *in vivo* scaling is the same for A and B.

To date, none of the published *in vitro* F_T values has been validated against or compared with *in vivo* F_T values due to the lack of clinical data generated with selective substrates or inhibitors. In facing this challenge, instead of relying on the data from transporter-specific clinical probes, a method has been developed in this study to estimate *in vivo* OATP1B1 F_T ($F_{T,OATP1B1}$) values by mechanistically modeling genotyped clinical pharmacokinetic data. The approach is based on the hypothesis that observed change in hepatic active uptake clearance due to OATP1B1 polymorphism depends on two factors: (1) the contribution of OATP1B1 to the hepatic active uptake clearance and (2) the change of OATP1B1-mediated intrinsic uptake activity by its polymorphism. Conversely, if the changes caused by genetic variations in both hepatic active uptake clearance and OATP1B1-mediated clearance are known, then the OATP1B1 contribution to the hepatic active uptake clearance can be calculated. The F_T values estimated from the new approach can be used to validate *in vitro* F_T methods, to generate transporter-specific scaling factors, and to build a foundation towards more accurate predictions for transporter-mediated clearance, DDI, and pharmacokinetic variability.

METHODS

Derivation of Equations

OATP1B1 is encoded by gene *SLCO1B1*. Multiple *SLCO1B1* single-nucleotide polymorphisms (SNPs) have been identified, among which the most studied SNP is c.521T>C in exon 5 (where c. represents coding DNA sequence, the number refers to the first nucleotide affected, and ">" indicates a substitution at DNA level) (15). The SNP c.521T>C reduces the OATP1B1 activity for certain substrates. As a result, compared with the wild-type OATP1B1 carriers (c.521TT), the mutation carriers (c.521CC) usually have reduced hepatic active uptake and increased systemic exposure. Both OATP1B1 and non-OATP1B1 mediated clearances ($CL_{OATP1B1}$ and $CL_{non\text{-}OATP1B1}$) contribute to the total hepatic active uptake ($CL_{active,uptake}$) of c.521TT groups (Eq. (1)). Since c.521T>C may not completely eliminate OATP1B1 activity, both $CL_{OATP1B1}$ and $CL_{non\text{-}OATP1B1}$ may also contribute to $CL_{active,uptake}$ of c.521CC groups (Eq. (2)).

$$CL_{active,uptake,521TT} = CL_{OATP1B1,521TT} + CL_{non-OATP1B1} \quad (1)$$

$$CL_{active,uptake,521CC} = CL_{OATP1B1,521CC} + CL_{non-OATP1B1} \quad (2)$$

The contribution of OATP1B1 in two groups is defined by its F_T value.

$$CL_{OATP1B1,521TT} = CL_{active,uptake,521TT} \cdot F_{T,OATP1B1,521TT} \quad (3)$$

$$CL_{OATP1B1,521CC} = CL_{active,uptake,521CC} \cdot F_{T,OATP1B1,521CC} \quad (4)$$

The two groups share the same $CL_{non\text{-}OATP1B1}$, assuming that c.521T>C does not change the activity of other transporters, and are not correlated with activity-changing SNPs of other transporters.

$$\begin{aligned} CL_{non-OATP1B1} &= CL_{active,uptake,521TT} \cdot \left(1 - F_{T,OATP1B1,521TT}\right) \\ &= CL_{active,uptake,521CC} \cdot \left(1 - F_{T,OATP1B1,521CC}\right) \end{aligned} \quad (5)$$

It is further assumed that the ratio of $CL_{OATP1B1,521TT}$ to $CL_{OATP1B1,521CC}$ is the same as the ratio of their intrinsic uptake activity (k_{521TT}/k_{521CC}).

$$\frac{CL_{OATP1B1,521TT}}{CL_{OATP1B1,521CC}} = \frac{k_{521TT}}{k_{521CC}} \quad (6)$$

Substituting Eq. (3) into Eq. (6) gives the following equations.

$$\frac{CL_{active,uptake,521TT} \cdot F_{T,OATP1B1,521TT}}{CL_{OATP1B1,521CC}} = \frac{k_{521TT}}{k_{521CC}} \quad (7)$$

$$CL_{OATP1B1,521CC} = CL_{active,uptake,521TT} \cdot F_{T,OATP1B1,521TT} \cdot \frac{k_{521CC}}{k_{521TT}} \quad (8)$$

Substituting Eqs. (5) and (8) into Eq. (2) leads to Eq. (9).

$$\begin{aligned} CL_{active,uptake,521CC} &= CL_{active,uptake,521TT} \cdot F_{T,OATP1B1,521TT} \cdot \frac{k_{521CC}}{k_{521TT}} \\ &\quad + CL_{active,uptake,521TT} \cdot \left(1 - F_{T,OATP1B1,521TT}\right) \end{aligned} \quad (9)$$

Rearrangement of Eq. (9) leads to Eq. (10), which calculates the $F_{T,OATP1B1}$ value in the c.521TT group based on the difference between hepatic active uptake clearance in c.521TT and c.521CC carriers (i.e., $CL_{active,uptake,521CC}/CL_{active,uptake,521TT}$) and the difference between intrinsic activity of c.521TT- and c.521CC-coded OATP1B1 (i.e., k_{521TT}/k_{521CC}).

$$F_{T,OATP1B1,521TT} = \frac{1-CL_{active,uptake,521CC}/CL_{active,uptake,521TT}}{1-k_{521CC}/k_{521TT}} \quad (10)$$

When the k_{521CC}/k_{521TT} ratio is not available, it is worth noting that this value is still lower bounded by zero. Alternatively speaking, k_{521CC}/k_{521TT} is a value between zero and one, depending on how much OATP1B1 activity is eliminated by c.521T>C mutation for a specific substrate. As such, although the $F_{T,OATP1B1,521TT}$ value cannot be calculated without the k_{521CC}/k_{521TT} ratio, its lower bound (i.e., the minimal contribution made by OATP1B1 to hepatic active uptake) can be estimated.

$$F_{T,OATP1B1,521TT} \geq 1-CL_{active,uptake,521CC}/CL_{active,uptake,521TT} \quad (11)$$

Rearrangement of Eq. (5) leads to Eq. (12).

$$\frac{CL_{active,uptake,521CC}}{CL_{active,uptake,521TT}} = \frac{1-F_{T,OATP1B1,521TT}}{1-F_{T,OATP1B1,521CC}} \quad (12)$$

Substituting Eq. (12) into Eq. (10) gives Eq. (13), which calculates the $F_{T,OATP1B1}$ value in the c.521CC group.

$$F_{T,OATP1B1,521CC} = \frac{F_{T,OATP1B1,521TT}\cdot k_{521CC}/k_{521TT}}{1-F_{T,OATP1B1,521TT}\cdot(1-k_{521CC}/k_{521TT})} \quad (13)$$

Without information about k_{521CC}/k_{521TT}, $F_{T,OATP1B1,521CC}$ can be any value between zero and $F_{T,OATP1B1,521TT}$.

Model Parameterization

In this study, k_{521CC}/k_{521TT} is approximated with *in vitro* k_{*15}/k_{*1a} published previously for a few compounds (16,17). The ratio of $CL_{active,uptake,521CC}$ to $CL_{active,uptake,521TT}$ is estimated by fitting systemic exposure data from c.521CC and c.521TT groups using a physiologically based pharmacokinetic (PBPK) model. A schematic diagram of the PBPK model structure and values of physiological parameters have been published previously (18) and are provided in the supplementary materials (Fig. S1 and Table S1). A few compound-specific parameters are fixed at their *in vitro* values during data fitting (Table S2 in the supplementary materials). Briefly, all tissues are connected with circulating blood. The compound distribution is perfusion limited in all tissues except for the liver. Tissue to plasma partition coefficients (K_P) in non-liver tissues are calculated with a published *in silico* method (19). Although drug transporters exist in some non-liver tissues (e.g., gut and kidney), the volume of these tissues are small compared with the liver. As such, assuming that the transport in these tissues does not significantly change the overall drug distribution, distribution into all non-liver tissues is approximated as perfusion limited to simplify the problem. The liver is modeled as permeability limited with five sequential segments. Every segment includes one pair of extracellular and intracellular sub-compartments. There are $CL_{active,uptake}$, passive diffusion clearance ($CL_{passive,diffusion}$) between extracellular and intracellular sub-compartments, and biliary excretion (CL_{bile}) or metabolism ($CL_{metabolism}$) within the intracellular sub-compartments. Different from the published model, an active hepatic basolateral efflux ($CL_{active,efflux}$), which actively transports compounds from the intracellular to the extracellular sub-compartments, is introduced into the liver model. To date, there is no solid data to show if the active basolateral efflux is involved in hepatic transport or not. This parameter is included to avoid making the arbitrary assumption that active basolateral efflux is not involved in hepatic transport. Absorption is described empirically using two sequential compartments with first-order absorption rates (k_a) and F_aF_g (i.e., the product of the fraction of a dose absorbed and the fraction of a drug passing though the gut wall without metabolism) in each compartment. The parameter values are assumed to be the same for the two compartments. Five sequential bile compartments with the same the first-order transfer rate (k_{bile}) are used to simulate the enterohepatic recirculation.

Additional to the ratio of $CL_{active,uptake,521CC}$ to $CL_{active,uptake,521TT}$, the following parameters are also estimated by fitting clinical data: $CL_{active,uptake,521TT}$, $CL_{passive,diffusion}$, $CL_{metabolism}$ (for atorvastatin, nateglinide, and repaglinide), CL_{bile} (for fexofenadine, pitavastatin, pravastatin, and rosuvastatin), k_a, F_aF_g, and k_{bile} (for fexofenadine, pitavastatin, pravastatin, and rosuvastatin). Although K_P are fixed at *in silico*–predicted values, a compound-specific scalar (S_{KP}) is estimated with other parameters. I assume that a single S_{KP} value apply to all non-liver tissues for a given compound. A fitted lag time (t_{lag}) in absorption is used whenever necessary. Pitavastatin is metabolized by glucuronosyltransferase in the liver, excreted into the bile, and potentially de-glucuronide and absorbed in the gut. To reduce the number of the fitted parameters in data fitting, hepatic metabolism of pitavastatin and biliary excretion of its metabolite are lumped into a single-parameter CL_{bile}, while de-glucuronidation and absorption are lumped into the oral absorption process described by k_a and F_aF_g.

During fitting, the data of an individual compound from different ethnic groups have been pooled together with dosing amount scaled to 10 mg. Values of $CL_{passive,diffusion}$ and S_{KP} are shared by different ethnic groups. The previous studies have shown that OATP1B1 activity is the same for different ethnic groups as long as they have the same *SLCO1B1* genotype (18,20). To include this observation into the data fitting, the following constraining condition is included in the model when data from more than one ethnic group are used. For example, when fitting data from Caucasian and Korean groups, the two groups have the same OATP1B1-mediated clearance for the same *SLCO1B1* genotype. However, the different ethnic groups may have different non-OATP1B1-mediated clearance and different total hepatic

active uptake clearance. This is because (1) unknown genetic variations may be associated with non-OATP1B1 transporters, (2) such variation may lead to increased or reduced activity for these non-OATP1B1 transporters, and (3) the frequency of the variations may be different in different ethnic groups. In Eqs. (14) and (15), $CL_{active,uptake}$ for Caucasian groups is split into $CL_{OATP1B1}$ and $CL_{non\text{-}OATP1B1}$, where $CL_{OATP1B1}$ is shared with other ethnic groups, while $CL_{non\text{-}OATP1B1}$ is a Caucasian-specific parameter. To simplify the problem, in the same ethnic group, non-OATP1B1-mediated clearance is assumed to be same.

$$CL_{active,uptake,521TT,Caucasian} = CL_{OATP1B1,521TT} + CL_{non-OATP1B1,Caucasian} \quad (14)$$

$$CL_{active,uptake,521CC,Caucasian} = CL_{OATP1B1,521CC} + CL_{non-OATP1B1,Caucasian} \quad (15)$$

Subtracting Eq. (15) from Eq. (14) leads to Eq. (16).

$$CL_{active,uptake,521TT,Caucasian} - CL_{active,uptake,521CC,Caucasian} = CL_{OATP1B1,521TT} - CL_{OATP1B1,521CC} \quad (16)$$

A similar equation can be derived for Korean groups.

$$CL_{active,uptake,521TT,Korean} - CL_{active,uptake,521CC,Korean} = CL_{OATP1B1,521TT} - CL_{OATP1B1,521CC} \quad (17)$$

Subtracting Eq. (16) from Eq. (17) leads to Eq. (18).

$$CL_{active,uptake,521TT,Caucasian} - CL_{active,uptake,521CC,Caucasian} = CL_{active,uptake,521TT,Korean} - CL_{active,uptake,521CC,Korean} \quad (18)$$

Rearranging Eq. (18) gives Eq. (19).

$$\frac{CL_{active,uptake,521CC,Korean}}{CL_{active,uptake,521TT,Korean}} = 1 - \frac{CL_{active,uptake,521TT,Caucasian} \cdot \left(1 - CL_{active,uptake,521CC,Caucasian} / CL_{active,uptake,521TT,Caucasian}\right)}{CL_{active,uptake,521TT,Korean}} \quad (19)$$

As such, in data fitting, the Korean ratio of $CL_{active,uptake,521CC}$ to $CL_{active,uptake,521TT}$ is not fitted directly but calculated using Eq. (19) with the values of the other three fitted parameters: Caucasian $CL_{active,uptake,521TT}$, Caucasian ratio of $CL_{active,uptake,521CC}$ to $CL_{active,uptake,521TT}$, and Korean $CL_{active,uptake,521TT}$. For fexofenadine, pravastatin, rosuvastatin, to improve the parameter identifiability, ungenotyped pharmacokinetic data following intravenous dosing from Caucasian subjects (21–23) are also included in fitting. The Caucasian intravenous data modeling shares all parameter values with genotyped Caucasian oral data modeling except for $CL_{active,uptake}$.

MATLAB is used for the modeling (Mathworks, Natick, MA, USA). Example MATLAB codes for rosuvastatin and a description for MATLAB analysis are provided in the supplementary materials. The global optimization with differential evolution and uncertainty analysis with Markov chain Monte Carlo (MCMC) for parameter estimation is performed as described before (18).

RESULTS

The model structure and optimized parameter values can reasonably describe the observed pharmacokinetic time course data in both *SLCO1B1* c.521TT and c.521CC groups (Fig. 1). Values of the fitted parameters are provided in the supplementary materials (Table S3). The minimal $F_{T,OATP1B1,521TT}$ values are estimated by fitting the genotyped pharmacokinetic data (Table I). It is worth noting that the minimal $F_{T,OATP1B1,521TT}$ values provided in Table I represent the lower bounds of possible $F_{T,OATP1B1,521TT}$ values. For example, in the Caucasian *SLCO1B1* c.521TT group, the minimal $F_{T,OATP1B1,521TT}$ is estimated to be 44.1% for atorvastatin, with a 95% confidence interval of 37 to 51%. It means that OATP1B1 contribution represents at least 44.1% of total hepatic active uptake of atorvastatin in the Caucasian *SLCO1B1* c.521TT group. The interval of 37 to 51% represents the uncertainty in estimating this lower bound by data fitting. Since 44.1% is only the lower bound of $F_{T,OATP1B1,521TT}$, it is possible that a larger portion of hepatic active uptake (e.g., 60%) is contributed by OATP1B1 in the Caucasian *SLCO1B1* c.521TT group. The minimal $F_{T,OATP1B1,521TT}$ estimated for pravastatin is associated with a relatively large uncertainty likely due to insufficient or non-ideal data in parameter estimation. Fexofenadine has a minimal $F_{T,OATP1B1,521TT}$ of 100%, indicating hepatic active uptake of this compound is probably completely mediated by OATP1B1.

With the ratio of activity between genotyped groups approximated using *in vitro* data, $F_{T,OATP1B1}$ values can be determined for atorvastatin, pitavastatin, pravastatin, and rosuvastatin in c.521CC and c.521TT groups (Table II). $F_{T,OATP1B1,521TT}$ and $F_{T,OATP1B1,521CC}$ values are usually similar for the same compounds among different ethnic groups. The estimated $F_{T,OATP1B1}$ values for pravastatin are different in Caucasian and Japanese groups. However, a large uncertainty is associated with pravastatin estimate in the Japanese group; hence, it is difficult to confidently compare this value with the corresponding Caucasian value.

DISCUSSION

This study describes a method to estimate hepatic transporter F_T using genotyped clinical pharmacokinetic data. Although the method is developed using *SLCO1B1* genotyped data, it can be applied to other transporters if data are available. The *in vivo* F_T values split lumped hepatic uptake clearance into individual clearance processes mediated by specific transporters, which will facilitate our understanding on transporter *in vitro* to *in vivo* extrapolation. The estimated $F_{T,OATP1B1}$ values in Table II can be used directly in the

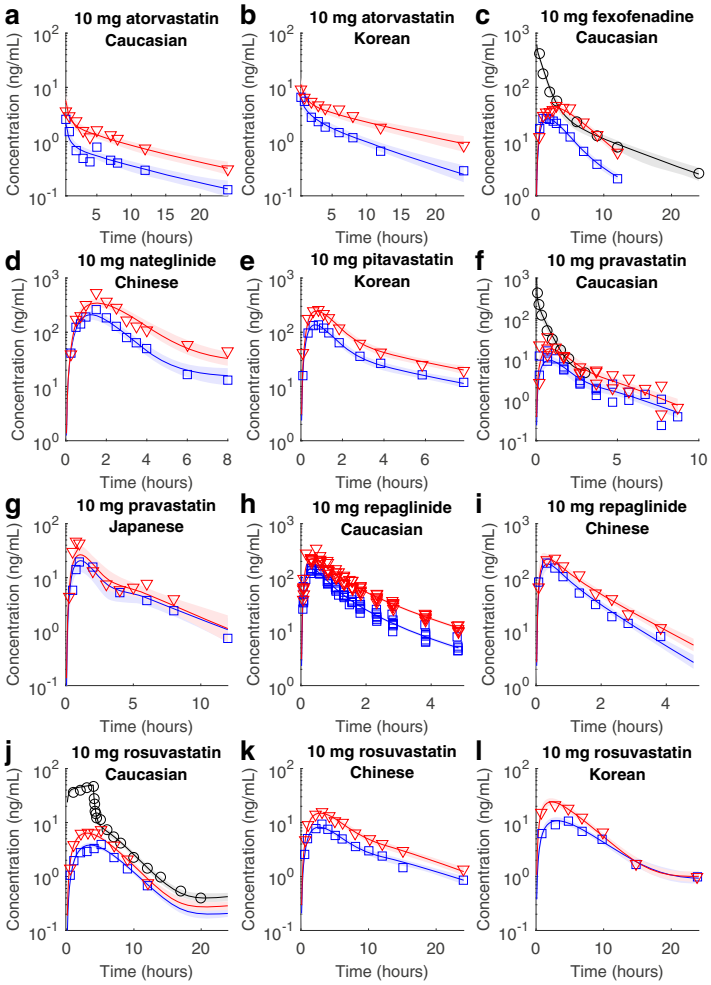

Fig. 1. Fitting systemic pharmacokinetic data of OATP1B1 substrates in *SLCO1B1* c.521TT and c.521CC groups. Red triangles and lines represent the observed and simulated plasma concentrations in c.521CC groups; blue squares and lines represent the observed and simulated plasma concentrations in c.521TT groups; and black circles and lines represent the observed and simulated plasma concentrations in the ungenotyped groups following intravenous dosing. The shaded areas represent 95% confidence intervals

previously established DDI prediction methods (5) as these compounds are widely used OATP1B1 clinical probes (40). For a few compounds analyzed in this study, the current study can only provide the minimal $F_{T,OATP1B1}$ in c.521TT

Table I. Estimated *In Vivo* Ratios of $CL_{active,uptake,521CC}$ to $CL_{active,uptake,521TT}$ and the Minimal $F_{T,OATP1B1,521TT}$

Compound	Ethnicity	Ratios of $CL_{active,uptake,521CC}$ to $CL_{active,uptake,521TT}$	Minimal $F_{T,OATP1B1,521TT}$ %	Reference of clinical data
Atorvastatin	Caucasian	0.559 (0.49–0.63)	44.1 (37–51%)	(24)
Atorvastatin	Korean	0.495 (0.38–0.63)	50.7 (37–62%)	(25)
Fexofenadine	Caucasian	1.04×10^{-8} (9.4×10^{-16}–0.029)	100 (97–100%)	(26)
Nateglinide	Chinese	0.389 (0.041–0.53)	61.1 (47–96%)	(27)
Pitavastatin	Korean	0.588 (0.52–0.66)	41.2 (34–48%)	(28)
Pravastatin	Caucasian	0.127 (3.0×10^{-10}–0.61)	87.3 (39–100%)	(29–31)
Pravastatin	Japanese	0.788 (0.14–0.99)	21.2 (0.91–98%)	(32)
Repaglinide	Caucasian	0.614 (0.59–0.64)	38.6 (36–41%)	(33–36)
Repaglinide	Chinese	0.749 (0.61–0.84)	25.0 (16–39%)	(37)
Rosuvastatin	Caucasian	0.638 (0.55–0.74)	36.2 (26–45%)	(24)
Rosuvastatin	Chinese	0.506 (0.42–0.59)	49.4 (41–58%)	(38)
Rosuvastatin	Korean	0.409 (0.28–0.57)	59.1 (43–71%)	(39)

Values in parentheses represent 95% confidence intervals

Table II. Estimated *In Vivo* $F_{T,OATP1B1,521TT}$ and $F_{T,OATP1B1,521CC}$

	Ethnicity	*In vitro* activity ratio of OATP1B1 *15 to *1a	$F_{T,OATP1B1,521TT}$ %	$F_{T,OATP1B1,521CC}$ %
Atorvastatin	Caucasian	0.22	56.5 (47–65%)	22.2 (16–29%)
Atorvastatin	Korean	0.22	65.0 (48–79%)	29.0 (17–46%)
Pitavastatin	Korean	0.17	49.6 (41–57%)	14.3 (10–19%)
Pravastatin	Caucasian	0.27	72.8 (42–97%)	41.5 (16–90%)
Pravastatin	Japanese	0.27	28.9 (1.1–100%)	9.69 (0.29–100%)
Rosuvastatin	Caucasian	0.027	37.2 (27–46%)	1.57 (0.97–2.3%)
Rosuvastatin	Chinese	0.027	50.7 (42–60%)	2.71 (1.9–3.9%)
Rosuvastatin	Korean	0.027	60.7 (44–73%)	4.00 (2.1–7.0%)

Values in parentheses represent 95% confidence intervals

population (Table I) due to limited data. If these minimal $F_{T,OATP1B1}$ values are used as guidance in projecting DDI risk, it is worth noting that the DDI risk increases with the increased $F_{T,OATP1B1}$ value in a nonlinear manner. For example, assuming that minimal $F_{T,OATP1B1}$ is 60% and AUC is mainly determined by hepatic active uptake, completely inhibiting OATP1B1 may result in an increased AUC by 3.3-fold if actual $F_{T,OATP1B1}$ is 70%, 5-fold if actual $F_{T,OATP1B1}$ is 80%, or 10-fold if actual $F_{T,OATP1B1}$ is 90%. The $F_{T,OATP1B1}$ values can also be used to predict pharmacokinetic variability due to genetic variations in ethnic groups (4). By replacing *in vitro* F_T with *in vivo* F_T, it helps to improve the prediction accuracy and reliability. One possible confounding factor in the current study, however, is the ratio of activity between genotyped groups approximated using *in vitro* data (e.g., k_{521CC}/k_{521TT} is approximated with *in vitro* k_{*15}/k_{*1a}). I cannot rule out the possible discrepancy between *in vitro* and *in vivo* ratios. The two SNPs c.521T>C and c.388A>G lead to four haplotypes, known as *1a (wild type, c.388A and c.521T), *1b (c.388G and c.521T), *5 (c.388A and c.521C), and *15 (c.388G and c.521C). In most c.521C>T genotyped pharmacokinetic studies, c.388A>G is not genotyped. As such, the c.521TT groups may include *1a/*1a, *1a/*1b, and *1b/*1b carriers, while c.521CC groups may include *5/*5, *5/*15, and *15/*15 carriers. The haplotypes *5 and *15 have relatively similar activity (17). However, *1b is potentially associated with a slightly reduced intrinsic activity but doubled protein expression level (17,41). Hence, the rate of the c.521T group may be underestimated while $F_{T,OATP1B1,521TT}$ may be overestimated. The assumption is hard to validate or invalidate with the data available so far because of (1) unknown proportions of *1a/*1a, *1a/*1b, and *1b/*1b carriers in individual pharmacokinetic studies which usually have small numbers of participants and (2) unclear effect of heterozygous *1a/*1b variation on transporter activity. For pravastatin, two published *in vitro* studies provide two different k_{*15}/k_{*1a} values: 0.41 from (16) and 0.12 from (17). Different *in vitro* studies result in different estimates for $F_{T,OATP1B1}$. The current study uses the average of the two published values. Future studies may be required to understand how to more accurately and precisely estimate the ratio of activity between genotypes using *in vitro* assay or other methods.

Nevertheless, $F_{T,OATP1B1}$ values estimated from the current approach using clinical data is still more credible than $F_{T,OATP1B1}$ completely based on *in vitro* data. To be a reasonable surrogate of *in vivo* $F_{T,OATP1B1}$, *in vitro* $F_{T,OATP1B1}$ values must be at least equal to or greater than the minimal $F_{T,OATP1B1}$ values reported in Table I, which are estimated without using *in vitro* ratio data. Using *in vitro* F_T in *in vivo* predictions, the published approaches assume that *in vitro* to *in vivo* scaling is similar across different hepatic uptake transporters. Furthermore, every *in vitro* F_T approach also has its additional assumptions. For example, all methods listed in Table III assume that high concentrations of substrates (or inhibitors) can completely inhibit all active uptake processes without causing toxicity issue. Some methods assume that scaling between OATP-transfected cells and hepatocytes can be accurately estimated with presumably selective substrates, while other methods assume that transporter activities are proportional to their expression levels. All such assumptions require further validation.

Table III. Published *In Vitro* $F_{T,OATP1B1}$

	Method 1	Method 2	Method 2	Method 3	Method 3
Reference	(7)	(8)	(11)	(8)	(11)
Atorvastatin			65% (Pooled lot PQP)		50% (Pooled lot PQP)
Pitavastatin	84% (HU4244) 98% (HU8085) 90% (HU8089)	100% (OCF) 69% (094) 85% (ETR)	42% (Pooled lot PQP)	360% (OCF) 110% (094) 170% (ETR)	32% (Pooled lot PQP)
Pravastatin			86% (Pooled lot PQP)		66% (Pooled lot PQP)
Rosuvastatin	72% (HU4244) 66% (HU8085) 64% (HU8089)		88% (Pooled lot PQP)		67% (Pooled lot PQP)

Human hepatocyte lot numbers are provided in parentheses

Table III summarizes previously developed *in vitro* F_T methods and results into three categories: method 1 (7), method 2 (8,11), and method 3 (8,11). In a plated human hepatocyte (PHH) assay, method 1 uses conditions with and without 500 μM atorvastatin to estimate the total active uptake processes and OATP1B1-selective siRNA to estimate $F_{T,OATP1B1}$. In a suspended human hepatocyte assay, method 2 assesses total active uptake with an approach similar to that of method 1. The relative activity factor (RAF) between OATP-transfected HEK cells and cryopreserved hepatocytes is estimated as the active uptake ratio between two assays using presumably OATP1B1- and 1B3-selective substrates estrone-3-sulfate and cholecystokinin octapeptide. OATP1B1-mediated hepatocyte uptake is predicted as the product of HEK active uptake and OATP1B1 RAF. The ratio of OATP1B1-mediated uptake to total active uptake in hepatocytes is believed to be $F_{T,OATP1B1}$. Method 3 is similar to method 2, except that RAF is replaced by the relative expression factor (REF). REF is estimated as the OATP expression ratio between hepatocytes and HEK cells. It is worth noting that in some early publications of methods 2 and 3, it is assumed that OATP1B1 and 1B3 are the only two transporters involved in hepatocyte active uptake; hence, the total active uptake is calculated as the sum of products of RAF (or REF) and active uptake rates in transfected HEK cells (8,9). This is a suspicious assumption that lacks validation. Essentially, based on Kunze and others' observation that the sum of OATP1B1- and 1B3-mediated uptake is less than the total uptake (11), this assumption is likely to be invalid. Hence, in Table III, instead of using the originally reported values, $F_{T,OATP1B1}$ is recalculated using the actual total active uptake observed in the hepatocyte.

With all caveats mentioned, *in vitro* methods can provide $F_{T,OATP1B1}$ values either greater than or very close to the minimal *in vivo* $F_{T,OATP1B1}$ values listed in Table I. Neither the results from different research groups using the same *in vitro* methods nor the results from the same research group using different methods completely agree with each other. The values reported by Kunze and others (11) have the best agreement with *in vivo* $F_{T,OATP1B1,521TT}$ values, although at least one of four compounds available for comparison still shows discrepancy. *In vitro* $F_{T,OATP1B1}$ values are compared with *in vivo* $F_{T,OATP1B1,521TT}$ (i.e. wild type) values, due to the fact that research teams usually pick the hepatocyte lot with high uptake activity, although no published *in vitro* studies mentioned above provides genetic information about hepatocyte lots. Using the REF method, data from Hirano and others lead to $F_{T,OATP1B1}$ values greater than one (8), possibly because the western blot method they used cannot accurately measures the transporter expression level. Unfortunately, there are only a few compounds for which both *in vitro* and *in vivo* $F_{T,OATP1B1}$ are available for comparison. Additional data are required in the future to further validate *in vitro* to *in vivo* correlation of $F_{T,OATP1B1}$.

CONCLUSION

In this study, a method is developed for the first time to estimate *in vivo* fractional contribution of OATP1B1 to total hepatic active uptake ($F_{T,OATP1B1}$) by mechanistically modeling genotyped clinical pharmacokinetic data. The estimation method and the estimated $F_{T,OATP1B1}$ values will facilitate our understanding and prediction on the transporter-mediated pharmacokinetics, DDI, and variability.

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