Research Article

Estimation of Ontogeny Functions for Renal Transporters Using a Combined Population Pharmacokinetic and Physiology-Based Pharmacokinetic Approach: Application to OAT1,3

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Abstract. To date, information on the ontogeny of renal transporters is limited. Here, we propose to estimate the in vivo functional ontogeny of transporters using a combined population pharmacokinetic (popPK) and physiology-based pharmacokinetic (PBPK) modeling approach called popPBPK. Clavulanic acid and amoxicillin were used as probes for glomerular filtration, combined glomerular filtration, and active secretion through OAT1,3, respectively. The predictive value of the estimated OAT1,3 ontogeny function was assessed by PBPK predictions of renal clearance (CL_R) of other OAT1,3 substrates: cefazolin and piperacillin. Individual CL_R post-hoc values, obtained from a published popPK model on the concomitant use of clavulanic acid and amoxicillin in critically ill children between 1 month and 15 years, were used as dependent variables in the popPBPK analysis. CL_R was re-parameterized according to PBPK principles, resulting in the estimation of OAT1,3-mediated intrinsic clearance (CL_{int,OAT1,3,invivo}) and its ontogeny. CL_{int,OAT1,3,invivo} ontogeny was described by a sigmoidal function, reaching half of adult level around 7 months of age, comparable to findings based on renal transporter-specific protein expression data. PBPK-based CL_R predictions including this ontogeny function were reasonably accurate for piperacillin in a similar age range (2.5 months–15 years) as well as for cefazolin in neonates as compared to published data (%RMSPE of 21.2 and 22.8%, respectively and %PE within ±50%). Using this novel approach, we estimated an in vivo functional ontogeny profile for CL_{int,OAT1,3,invivo} that yields accurate CL_R predictions for different OAT1,3 substrates across different ages. This approach deserves further study on functional ontogeny of other transporters.

KEY WORDS: Pediatrics; physiology-based PK; Population PK; OAT1,3; ontogeny.

INTRODUCTION

Pediatric renal clearance (CL_R) is driven by physiology related changes to kidney size, number of glomeruli and nephron filtration capacity, renal blood flow, expression of drug binding plasma proteins and expression of transporters. Throughout the pediatric age-range, the maturation of glomerular filtration rate (GFR) has been extensively studied by various groups (1–7), however, less is known about the functional in vivo development of other processes contributing to CL_R (8) such as active tubular secretion (ATS), which is mediated through transporters in the kidneys. In vivo transporter activity cannot be directly quantified but has to be derived from other measures. Recently, the ontogeny of individual renal transporters has been quantified by measuring transporter-specific protein expressions in postmortem kidney samples from children of different ages (9). However, there is limited information about how protein expression relates to in vivo transporter activity and whether
Quantifying the Ontogeny Function of OAT1,3 In Vivo

Clinical studies showed that the majority of an amoxicillin and clavulanic acid dose is recovered unchanged in urine (15-18) and in vitro evidence suggests that active secretion of amoxicillin is mainly mediated through OAT3 and to a lesser extent by OAT1 (13, 19). Different minor elimination routes may be involved, yet here we assume the clinical data to reflect the major elimination routes only. This implies the assumption that clavulanic acid clearance through other elimination routes than GF mature at the same rate as GF. For amoxicillin the extent of clearance through elimination routes other than active tubular secretion is assumed to be the same as for clavulanic acid and the difference in clearance between these two drugs is fully attributed to active tubular secretion through OAT1/3. Finally, even though the OAT1/3 transporter works in tandem with MRP4 efflux transporters, the contribution of MRP4 transporters to the CL_R of amoxicillin and for piperacillin and cefazolin, mentioned later in the “methods” section, was excluded in the current example as the expression of this transporter was found to remain constant with age (9).

Individual post-hoc CL_R values for clavulanic acid and amoxicillin in pediatric patients were obtained from a population PK model of De Cock et al. (20). In short, a simultaneous popPK analysis was performed for both drugs based on data obtained after the administration of a fixed dose ratio of 1:10 (clavulanic acid:amoxicillin) in 50 intensive care pediatric patients with ages between 1 month and 15 years (median age of 2.6 years) (20). The PK of clavulanic acid and amoxicillin were described by a two- and a three-compartment model, respectively, with inter-individual variability (IVV) on renal clearance (CL_R) and central volume of distribution. The covariate analysis identified current weight as a statistically significant predictor for the IVV on both central volume of distribution and CL_R, whereas vasopressor treatment and cefazolin C were found to be statistically significant predictors only for the IVV on CL_R (20).

In a sequential step, CL_R was re-parameterized according to PBPK principles to reflect clearance through glomerular filtration (CL_GF) and through active tubular secretion (CL_ATS) (Eqs. 1 and 2) (21). The PBPK-based model for CL_R assumes a serial arrangement for GF and ATS, in which CL_R of clavulanic acid was described by CL_GF only (CL_ATS = 0), while CL_R of amoxicillin was described by a combination of CL_GF and CL_ATS.

\[
CL_R = CL_GF + CL_ATS = (GFR \times f_u) \times \left(\frac{Q_u \times GFR \times f_u \times CL_{ATS}}{Q_u + f_u \times CL_{ATS}}\right) / BP
\]

(1)

\[
CL_{ATS} = CL_{ATS, invivo} \times om(OAT3) \times PTCPGK \times KW
\]

(2)

In equation 1, GFR stands for glomerular filtration rate, f_u for drug fraction unbound, Q_u for renal blood flow, CL_{sec,OAT1,3} for secretion clearance through OAT1,3, and BP for blood to plasma ratio. Equation 2 shows how CL_{sec,OAT1,3} is obtained by multiplying CL_{int,OAT1,3, invivo} that
stands for OAT1,3-mediated in vivo intrinsic clearance in adults, with \( \text{ont}_{\text{OAT1,3}} \) that stands for the ontogeny function for OAT1,3, PTCPGK that stands for proximal tubule cells per gram kidney, and KW that stands for kidney weight in grams.

The adult PBPK-based model for CL-R through a combination of GF and ATS (Eqs. 1 and 2) was extrapolated to the pediatric population. For this, published functions that describe the age-related changes of the system-specific parameters (i.e., GFR (2), renal blood flow (22), and kidney weight (22)) and of the drug-specific parameters impacted by changes in system-specific parameters (i.e., serum albumin concentrations (4) that influence the fraction unbound (23), and hematocrit levels that influence BP (22)) were inputted, as shown in Table S1. Values for \( f_u \) (24) and BP_{amox} (25) as reported in adults were used (\( f_u \text{clav}_{\text{acid}} = 0.75; f_u \text{amox} = 0.82; \) BP_{amox} = 0.55). \( \text{CL}_{\text{int,OAT1,3,in vivo}} \) reflects both the expression and activity of the OAT1,3 transporter in adults. Assuming PTCPGK to remain constant at adult values, this only leaves \( \text{CL}_{\text{int,OAT1,3,in vivo}} \) and its ontogeny function (\( \text{ont}_{\text{OAT1,3}} \)) to be estimated. This was done using the individual CL-R values from the population model as dependent variables and deriving the system-specific PBPK parameters based on the individual patient characteristics for each patient.

Pediatric typical CL-GF values were obtained using a published GFR maturation function developed for children with a normal renal function (2). However, when compared to normal CL-GF values, CL-R of both drugs as estimated with the population PK models, were found to be increased in the critically ill children included in the dataset of the current analysis (20). Hence, the PBPK-based re-parameterization of CL-GF included a typical GF correction factor (\( \theta_{\text{corr}} \)) with IIV (\( \eta_{\text{GFR}} \)) to account for this difference (equation 3).

\[
\text{CL}_{\text{R,clavulanic acid},i} = \text{GFR} \times f_u\text{clav}_{\text{acid}} \times \theta_{\text{corr}} \times e^{\eta_{\text{GFR}}} \tag{3}
\]

As both amoxicillin and clavulanic acid were administered simultaneously to each child, from the data on clavulanic acid the GF correction factor and IIV on GFR for each pediatric patient was estimated. According to Eqs. 4 and 5, the difference between the individual values for CL-R of amoxicillin and CL-R of clavulanic acid were used to estimate CL-ATS, which was the basis for the estimation of the IIV on the in vivo \( \text{CL}_{\text{sec,OAT1,3}} \) value and subsequently the OAT1,3 ontogeny function (\( \text{ont}_{\text{OAT1,3}} \)).

\[
\text{CL}_{\text{R,amoxicillin},i} = \text{GFR} \times f_u\text{amox} \times \theta_{\text{corr}} \times e^{\eta_{\text{GFR}}} + \frac{(Q_R - \text{GFR}) \times f_u\text{amox} \times CL_{\text{sec,OAT1,3},i}}{Q_R + f_u\text{amox} \times \frac{CL_{\text{sec,OAT1,3},i}}{BP_{\text{amox}}}} \tag{4}
\]

\[
\text{CL}_{\text{sec,OAT1,3},i} = \theta_{\text{CLint,OAT1,3,in vivo}} \times e^{\theta_{\text{TL50,OAT1,3,in vivo}}} \times \text{ont}_{\text{OAT1,3}} \times \text{PTCPGK} \times \text{KW} \tag{5}
\]

To quantify the ontogeny profile of CL_{\text{int,OAT1,3,in vivo}} different covariates (i.e. postnatal age, postmenstrual age, weight) were explored using sigmoid relationships (Eq. 6) or a simplification of this equation (i.e., an exponential equation). In Eq. 6, \( \text{hill} \) is the hill coefficient, which quantifies the steepness of the ontogeny slope and \( TM_{50} \) quantifies the age at which OAT1,3 reaches half of the adult value.

\[
\text{ont}_{\text{OAT1,3}} = \frac{\text{COV}_{\text{hill}}}{\text{COV}_{\text{hill}} + TM_{50}^{\text{hill}}} \tag{6}
\]

The statistical significance of including the \( \text{ont}_{\text{OAT1,3}} \) function in the equation for CL_{\text{sec,OAT1,3,i}} to obtain CL-R of amoxicillin was assessed according to the likelihood ratio test on the difference in objective function value. Under the assumption of a \( \chi^2 \) distribution, the objective function value of a model with one more degree of freedom had to be 3.84 points lower, with a corresponding \( p < 0.05 \) to indicate statistical significance (26). For graphical goodness-of-fit, a plot was made to check for prediction bias of the individual CL-R values obtained either with the PBPK model or the individual post hoc values from the population PK model that served as the dependent variable in these fits. In addition, ETA (\( \eta_{\text{GFR}}, \text{ont}_{\text{OAT1,3,in vivo}} \)) vs. covariate plots (age, weight) are made to check for structural accuracy in PK parameters.

**Predictive Properties of the OAT1,3 Ontogeny Function for New Substrates**

To assess the predictive performance of the obtained OAT1,3 maturation function, the PBPK model that includes the estimated ontogeny function for OAT1,3 (Eqs. 1 and 2) was used for pediatric PBPK CL-R predictions of piperacillin and cefazolin, two other substrates of the OAT1,3 transporter. PBPK predictions of CL-R were compared to published typical pediatric CL-R predictions by population PK models of the same drugs. Population models are considered the gold standard for deriving CL-R values from observed concentration-time data and since neither the PBPK model nor the typical predictions by a population PK model take random inter-individual deviations in CL-R into account, they can be directly compared.

To obtain the pediatric PBPK predictions for CL-R, we collected literature values for \( f_u \_\text{adult} \) of 0.8 (27) and 0.31 (25) for piperacillin and cefazolin, respectively, and for BP adult of 0.55 for both drugs. CL_{\text{int,OAT1,3,in vivo}} in Eq. 2 had to be derived for both drugs. This was done based on published in vitro activity data as measured in assays with OAT1,3 transfected cells (1.95 \( \mu \text{g/mg protein} \) (27) and 7.1 \( \mu \text{g/mg protein} \) (25) for piperacillin and cefazolin respectively). These values were further optimized based on the in vivo adult values for CL_{\text{int,OAT1,3}} using a retrospective IVIVE approach. More details on the retrospective IVIVE are provided in the supplemental materials.

The drug-specific CL_{\text{int,OAT1,3,in vivo}} values obtained in the retrospective IVIVE step were used in Eqs. 1 and 2 of the renal PBPK model to obtain pediatric CL-R predictions for cefazolin and piperacillin. Pediatric PBPK CL-R predictions
for piperacillin and cefazolin were made for typical individuals with the same demographic characteristics as the individual patients reported in the original publications describing the pediatric population PK models of these drugs (20, 28). This means that, for piperacillin, PBPK $CL_{R}$ values were estimated for 47 pediatric patients with ages between 2.5 months and 15 years (median age of 2.83 years). For cefazolin, the PBPK $CL_{R}$ values were estimated for 26 near-term neonates with gestational age higher than 35 weeks and postnatal age (PNA) between 1 and 30 days (median of 8 days). For this, the OAT3 ontogeny function obtained above for children of 1 month and older based on data from clavulanic acid and amoxicillin was extrapolated to the neonatal population.

Pediatric PBPK $CL_{R}$ predictions were visually and quantitatively compared to typical estimates obtained with published population PK models for these two OAT1,3 substrates. Precision was quantified as percentage root mean square prediction error ($%\text{RMSPE}$) (Eq. 7) and bias as percentage prediction error ($%\text{PE}$) (Eq. 8).

$$\frac{1}{N} \times \sum_{i=1}^{N} \left( \frac{CL_{R,\text{PBPK}} - CL_{R,\text{reference}}}{CL_{R,\text{reference}}} \right) ^2 \times 100, \quad (7)$$

$$%\text{PE} = \left( \frac{CL_{R,\text{PBPK}} - CL_{R,\text{reference}}}{CL_{R,\text{reference}}} \right) \times 100, \quad (8)$$

In both equations, $CL_{R,\text{PBPK}}$ are the $CL_{R}$ predictions obtained with the renal PBPK model in pediatrics and $CL_{R,\text{reference}}$ represents the $CL_{R}$ values for typical $CL_{R}$ predictions obtained with the published population PK models (28, 29). $%\text{RMSPE}$ and $%\text{PE}$ were calculated separately for piperacillin and cefazolin and reported overall as well as per age group. $CL_{R,\text{PBPK}}$ was considered to be accurately predicted if $%\text{RMSPE}$ and $%\text{PE}$ was within ±30%, reasonably accurately predicted between −30−50% and 30−50% and inaccurate when $%\text{RMSPE}$ and $%\text{PE}$ were outside ±50%. Note that $%\text{RMSPE}$ can only take positive values.

**RESULTS**

**Quantifying the Ontogeny Function of OAT1,3**

With the popPBPK approach, $CL_{GF}$ was separated from $CL_{ATS}$ such that $CL_{\text{int},\text{OAT1,3,in vivo}}$ and its ontogeny profile could be estimated in children as young as 1 month up to 15 years of age. Figure 1 shows the ontogeny profile of OAT1,3 as best described by a sigmoidal relationship based on PNA. $CL_{\text{int},\text{OAT1,3,in vivo}}$ was estimated to be 15.8 ml/h/g kidney (RSE% of 5%) at 15 years with an IV of 78.5%. This high IV suggests large differences between individual values obtained for $CL_{\text{int},\text{OAT1,3,in vivo}}$. $CL_{\text{int},\text{OAT1,3,in vivo}}$ was found to reach half of the adult capacity at a PNA of 27.3 weeks (RSE of 28%), which is around 7 months. The rapid ontogeny of OAT1,3 was captured by a hill exponent of 1.17 (%RSE of 36%). The estimated transporter ontogeny fractions range from 0.1 at 1 month and 1 at 15 years. The GF correction factor used to account for the increased $CL_{R}$ in critically ill children was estimated at 1.83 (RSE of 4%) with an IV of 24.4%.

The goodness-of-fit plots did not show any bias for $CL_{R}$ predictions obtained with $CL_{R}$ re-parameterized according to PBPK principles. Neither Fig. S1, which depicts popPBPK $CL_{R}$ predictions vs. the popPK $CL_{R}$ predictions, nor Fig. S2, which depicts the $\eta_{GF}$ and $\eta_{\text{CL\text{int},OAT1,3,in vivo}}$ vs. covariates (i.e., weight and age) show any bias. This suggests that the PBPK-based re-parameterization as $CL_{GF}$ (Eq. 3) can predict individual clavulanic acid $CL_{R}$ values accurately and that the reparameterization for $CL_{ATS}$ together with $CL_{\text{ATS}}$ (Eq. 4) can accurately predict the $CL_{R}$ of amoxicillin as excreted by GF and ATS through OAT1,3.

Figure 2 shows the total $CL_{R}$ for amoxicillin and the contribution of $CL_{GF}$ and $CL_{ATS}$ to $CL_{R}$ for each individual. Total $CL_{R}$ increases almost 7-fold between neonates younger than 1 year and children of 10 years and older (median of 1.64 L/h and 12 L/h, respectively). The median contribution of ATS to amoxicillin $CL_{R}$ for the studied pediatric population was 22% (range: 4–40%). Even if variability in ATS contribution was high within groups of individuals with similar ages, the ATS contribution increased with age, on average, from 14% in children younger than 1 year to 18% in children of 1–2 years, 21% for children of 2–5 years, 24% for children 5–10 years, reaching 29% for children older than 10 years.

**Fig. 1.** Ontogeny function for OAT1,3-mediated intrinsic clearance normalized by kidney weight ($CL_{\text{int},\text{OAT1,3,in vivo}}$—blue line) described by a sigmoidal function based on age and displayed throughout the studied pediatric age-range (1 month to 15 years), on a double-log scale. The orange dots represent the individual secretion clearance estimates normalized by kidney weight. See Eq. [5] for more details.
Predictive Properties of the OAT1,3 Ontogeny Function

Figure 3 shows the pediatric CL_R predictions for piperacillin and cefazolin obtained with the PBPK-based model and the identified OAT1,3 ontogeny function based on clavulanic acid and amoxicillin overlaid with the typical clearance estimates obtained with the published population PK models. The %RMSPE calculated between PBPK CL_R and typical CL_R predictions for piperacillin (Fig. 3a) over the entire age-range (2.5 months to 15 years) was 21.8% with a %PE interval between −33.2% and 25.4%. When stratified per age groups (i.e., younger than 1 year, 1–2 years, 2–5 years, 5–10 years and older than 10 years) %RMSPE is generally higher for children under 5 years (23.3, 22.2, and 27.4% vs. 14.9, 18.8%). For neonates (Fig. 3b), the %RMSPE calculated between PBPK CL_R and typical CL_R predictions for cefazolin was 22.2% with %PE interval between −34.4 and 46%.

For both pediatric populations the PBPK-based CL_R predictions can be considered reasonably accurate with %RMPE < 30% and %PE within ±50%. For piperacillin, the PBPK-based CL_R predictions tend towards overprediction (Fig. 3a), with all %PE values below 0%, although percentage deviations were acceptable [%PE between −13.3 and −28.8%] for children older than 1 year. For cefazolin in neonates, predictions are reasonably accurate (Fig. 3b), with PBPK-based CL_R predictions tending towards underprediction [%PE between 18.1 and 46%] for children older than 10 days.

DISCUSSION

With a combined population PK with PBPK approach, referred to as popPBPK, we estimated the functional in vivo ontogeny profile for OAT1,3, a parameter that cannot be obtained through direct measurements, down to the age of 1 month. Under the assumption that clavulanic acid is entirely eliminated through GF and amoxicillin through GF and ATS through OAT1,3, we used clinical PK data of children that received both drugs at the time to define a maturation function for ATS through OAT1,3. Using a population PK approach, we derived the individual CL_R values for both drugs that served as dependent variable for the popPBPK approach. CL_R was re-parameterized according to PBPK principles to take advantage of existing information about drug- and system-specific properties while estimating the ontogeny of OAT1,3 in vivo and the variability on GFR and on OAT1,3-mediated intrinsic clearance in vivo (CL_{int,OAT1,3} in vivo).

Our group recently developed a PBPK simulation framework for investigating the impact of ontogeny of renal secretion transporters on CL_R by predicting pediatric CL_R for hypothetical drugs with an array of drug properties (30). By looking at the difference between PBPK CL_R predictions with or without inclusion of the ontogeny function, probe drugs for quantifying the ontogeny of transporters were identified. According to the findings with this framework, amoxicillin, which has an estimated CL_{int,OAT1,3} in vivo of 4.4 μl/min/mg protein and a f_u of 0.82 (31), has the potential of serving as a probe to quantify OAT1,3 ontogeny. Furthermore, the clinical data available for probe drugs for GF and a combination of GF and ATS (clavulanic acid and amoxicillin, respectively) administered to the same individuals was paramount to separate between these two processes.

OAT1,3 ontogeny for the OAT1,3-mediated intrinsic clearance is steep in the first year of life, attaining half of the adult value around 7 months of age. This estimated ontogeny function was included in the pediatric PBPK-based model for CL_R through GF and ATS. Even though the functional in vivo OAT1,3 ontogeny profile was derived from clinical data obtained in critically ill patients without renal dysfunction, it predicted the CL_R for other drugs that are substrates for OAT1,3 reasonably accurate, as compared to popPK CL_R predictions for these drugs. Assuming clearance to be only mediated by GF and ATS, for piperacillin the PBPK CL_R predictions over an age-range of 2.5 months to 15 years lead to a %RMSPE of 21.8% [%PE: −33.2–25.4%] with a trend towards under-prediction for children older than 1 year. For cefazolin, extrapolation of CL_R predictions to near term neonates with ages between 1- and 30-days lead to a %RMSPE of 22.2% [%PE: −34.4–46%], with a trend towards under-prediction for children older than 10 days.

Previously, Hayton et al. used para-aminohippurate to derive an ontogeny profile of undifferentiated active renal secretion in vivo, concluding that 50% maturation is achieved around 1 year of age (3), which is comparable with our findings. Recently, more insight into differentiated ontogeny profiles of individual renal transporters have been quantified based on direct measurements of the expression of transporter-specific proteins in kidney samples taken postmortem from children of various ages, as described in detail by Cheung et al. (9). This group characterized the ontogeny of OAT1,3 as a sigmoidal function based on PNA in weeks with children reaching half of the adult values around 8 months of age (T_{M50} = 30.7 weeks [95% CI: 16.64–50.97]) and the steepness of the ontogeny slope given by a hill coefficient of 0.51 (95% CI: 0.35–0.71). While our findings align with Cheung et al. regarding the age at which half of the adult level is reached, which was estimated to be around 7 months with our function, we found a steeper ontogeny for OAT1,3, as shown by a 2-fold higher estimated hill coefficient. The impact of these differences on the ontogeny profiles is illustrated in Fig. 4. This figure shows relatively similar OAT1,3 ontogeny found by both methods at ages above the T_{M50} values, but for younger ages the function quantified in our work shows lower ontogeny values. Given the low number of observed values at these younger ages in both analyses, the uncertainty around the ontogeny below 7 months of age is high for both analyses. More data are required to establish the accuracy of the estimated in vivo functional ontogeny profile in the first year of life.

Although drugs may be predominantly eliminated by a particular pathway, they are rarely exclusively cleared through a single, well-defined pathway. However, despite the fact that the estimated functional OAT1,3 ontogeny profile may be impacted by minor elimination pathways contributing to the clearance of clavulanic acid and amoxicillin, this function could be used to obtain accurate pediatric PBPK-based CL_R predictions for two other drugs that are predominantly, though not exclusively, eliminated through GF and OAT1,3-mediated ATS, namely piperacillin and cefazolin. Despite small trends towards over and under-
prediction respectively, CL_{R} predictions for piperacillin and cefazolin were reasonably accurate with %RMSPE of 21.8 and 22.2%, which is well below the 2-fold error, which is the generally accepted criterion for accuracy of PBPK predictions. The tendency towards over-prediction of pediatric PBPK CL_{R} for piperacillin could be explained by other processes involved in renal elimination that are not accounted for in the PBPK model. It could for instance be that there is passive or active reuptake of these drugs in the kidneys. Alternatively, the authors of the popPK model that served as the reference values, reported a (temporary) impairment of the renal maturation function (29) which could explain the lower CL_{R} values obtained with the popPK model as compared to the PBPK CL_{R} predictions, the latter of which does not take (potential) renal impairment into account. A second drug, cefazolin, was used to assess the accuracy of this function for extrapolations to term newborns below 1 month of age. Remarkably, despite a small trend towards under-prediction of CL_{R} values for cefazolin in part of the newborns, all predictions can still be considered accurate.

The methodology proposed here is the first to enable the assessment of functional in vivo activity, rather than mRNA

Fig. 2. Contribution of clearance through glomerular filtration (CL_{GF} – bottom blue boxes) and through active tubular secretion (CL_{ATS} – top orange boxes) to total renal clearance of amoxicillin (CL_{R} – sum of blue and orange boxes) for each pediatric patient of the studied population sorted and grouped by age. The numbers in each box show the relative contribution of CL_{GF} and CL_{ATS} to total CL_{R} for each individual

Fig. 3. Renal clearance (CL_{R}) of piperacillin (a) and cefazolin (b) versus age in pediatric patients in children (a) and neonates (b). The pediatric PBPK CL_{R} predictions (dark blue) are overlaid with the typical CL_{R} estimates obtained with the published population pharmacokinetic model (orange)
or transporter expression or \textit{ex vivo} activity. As such it cannot
only augment the currently available methods to study renal
transporter maturation throughout the pediatric age-range, but
can also offer a valuable new dimension to this research.
Essential in our approach is the requirement of data on two
probe drugs that are predominantly excreted by specifically
GFR and a combination of GFR and ATS through a specific
transporter. As studies in healthy pediatric populations are
not allowed, the two probe drugs would have to be regularly
prescribed for therapeutic purposes in children across the
entire age-range. Furthermore, practical and ethical con-
straints may require assumptions to be made in the imple-
mentation of this method. For instance, in the example used
here to illustrate our approach, we assumed exclusive
elimination of our probe drugs through GF and OAT1,3-
mediated ATS, as ethical and practical constraints prevent
urine collection over prolonged durations and thereby
the formal assessment of the contribution of renal excretion to
overall drug elimination. This may have impacted the
accuracy of the obtained ontogeny function, but it does not
impact our proposed methodology conceptually. If informa-
tion on minor elimination routes would become available, this
could be included in the PBPK model to further refine the
estimated ontogeny function.

**CONCLUSION**

The ontogeny of functional \textit{in vivo} OAT1,3 activity was
derived by using a combined population PK and PBPK
modeling approach. This popPBPK approach leverages the
knowledge on underlying physiological processes included in
PBPK models and information carried by individual PK
parameters as quantified with a population approach, to
derive parameters that cannot be measured \textit{in vivo}. With this
methodology we derived the renal OAT1,3 transporter
ontogeny \textit{in vivo}. This ontogeny function was included in
the pediatric PBPK-based model $\text{CL}_R$ for two other OAT1,3
substrates and on average predicted $\text{CL}_R$ throughout
the entire pediatric age-range accurately. This methodology
could be applied to other transporters substrates to characterize the
\textit{in vivo} ontogeny of the remaining renal transporters to
further increase our understanding on renal development and
increase the accuracy in predicting pediatric $\text{CL}_R$.

**SUPPLEMENTARY INFORMATION**

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