The role of glomerular filtration and active tubular secretion in predicting renal clearance of drugs in children using population pharmacokinetic and physiology-based pharmacokinetic modeling approaches: unspinning the yarn

Cristea, S.

Section IV. Ontogeny of renal transporters and its impact on drug renal clearance in children

Chapter 5

The influence of drug properties and ontogeny of transporters on pediatric renal clearance through glomerular filtration and active secretion - a simulation-based study

S Cristea, EHJ Krekels, A Rostami-Hodjegan, K Allegaert, CAJ Knibbe

AAPS J 22, 87 (2020).
Epub ahead of print, doi.org/10.1208/s12248-020-00468-7

5.1 Abstract

Glomerular filtration (GF) and active tubular secretion (ATS) contribute to renal drug elimination, with the latter remaining understudied across the pediatric age-range. Therefore, we systematically analyzed the influence of transporter ontogeny on the relative contribution of GF and ATS to renal clearance CL_R for drugs with different properties in children.

A physiology-based model for CL_R in adults was extrapolated to the pediatric population by including maturation functions for the system-specific parameters. This model was used to predict GF and ATS for hypothetical drugs with a range of drug-specific properties, including transporter-mediated intrinsic clearance ($CL_{int,T}$) values, that are substrates for renal secretion transporters with different ontogeny patterns. To assess the impact of transporter ontogeny on ATS and total CL_R, a percentage prediction difference (%PD) was calculated between the predicted CL_R in the presence and absence of transporter ontogeny.

The contribution of ATS to CL_R ranges between 41% and 90% in children depending on fraction unbound and CLint,T values. If ontogeny of renal transporters is <0.2 of adult values, CL_R predictions are unacceptable (%PD > 50%) for the majority of drugs regardless of the pediatric age. Ignoring ontogeny patterns of secretion transporters increasing with age in children younger than 2 years results in CL_R predictions that are not systematically acceptable for all hypothetical drugs (%PD>50% for some drugs).

This analysis identified for what drug-specific properties and at what ages the contribution of ATS on total pediatric CL_R cannot be ignored. Drugs with these properties may be sensitive in vivo probes to investigate transporter ontogeny

5.2 Introduction

Between 21% and 31% of marketed drugs are primarily renally cleared [1]. Processes underlying renal clearance (CL_R) include glomerular filtration (GF), active tubular secretion (ATS), reabsorption and renal metabolism. Maturation of GF has been extensively studied and quantified in children. However, less is known about the impact of maturation in the other process on CL_R, partly due to the lack of specific biomarkers to distinguish between the activity of different transporters and to the overlap in specificity of transporters for different substrates. Together with GF, ATS is one of the major contributing pathways for CL_R, ontogeny of ATS is therefore the focus of the current analysis.

ATS involves different transporter systems located on the basolateral and apical sides of the proximal tubule cells of the kidney. These systems enable the efflux of drugs from the blood into the tubule where pre-urine is formed [2]. The expression of renal transporters was found to change in children [3]. However, these findings are based on a limited number of postmortem kidney samples collected throughout the pediatric age-range [3]. Furthermore, there is limited information about the relationship between transporter-specific protein expression and transporter activity [4] or whether this remains constant with age. Finally, the extent to which transporter activity impacts ATS and subsequently total CL_R has not been quantified yet for the pediatric population.

Physiology-based pharmacokinetic (PBPK) models [5] integrate prior knowledge on drug- and system-properties. This configuration can be leveraged to perform extrapolations to unstudied scenarios. For example, PBPK models can be back-extrapolated to the pediatric population by taking into account the developmental changes in system-parameters and be further used to make predictions in this special population for drugs that have not been studied in children yet. Previously, our group has used PBPK approaches in an innovative manner to systematically assess in which situations empirical scaling methods (i.e. allometric scaling, linear scaling) could be used to accurately scale plasma clearance of drugs that were eliminated by hepatic metabolism or GF for a broad range of hypothetical drugs [6,7]. However, due to limited information on the ontogeny of renal transporters, the accuracy of clearance

scaling for drugs eliminated through ATS could not be addressed.
Using a similar PBPK-based modelling approach as the one described above, we performed a systematic analysis to investigate the impact of the ontogeny of renal secretion transporters in relation with maturation of other physiological processes on the relative contribution of GF and ATS to CL_R as well as on the total CL_R. This assessment was performed throughout the pediatric age-range for a large number of hypothetical drugs with different properties covering a realistic parameter space. Moreover, to assess the impact of renal transporter ontogeny on CL_R throughout the pediatric population, we compared CL_R predictions obtained with and without including ontogeny patterns for renal transporters.

5.3 Methods

5.3.1 Expansion of a PBPK framework to predict CL_R in children
For this simulation study, a PBPK-based framework was developed analogue to the one published by Calvier et al. for plasma clearance by liver metabolism and GF [6]. R v3.5.0 under R studio 1.1.38 was used to build the framework and to perform the systematic simulations.

An existing PBPK model for predicting CL_R in adults [5] was extrapolated to the pediatric population by incorporating published maturation functions for the system-specific parameters in the model. The model assumes a serial arrangement of the two major contributing pathways, GF and ATS (equation 1).

$$CL_R = CL_{GF} + CL_{ATS} = f_u \times GFR + \frac{(Q_R - GFR) \times f_u \times CL_{int,sec}}{Q_R + f_u \times \frac{CL_{int,sec}}{BP}} \quad [1]$$

where CLGF and CLATS represent the clearance by GF and ATS, respectively and f_u is the fraction unbound, GFR is the glomerular filtration rate, Q_R is renal blood flow, BP is the blood to plasma ratio of the drug, and $CL_{int,sec}$ is the intrinsic secretion clearance of the active transporters. This model assumes that only the unbound drug in plasma is available for elimination whereas drugs bound to plasma proteins or accumulated in erythrocytes are considered unavailable for elimination.

Maturation functions from literature were included for plasma concentrations of human serum albumin (HSA) and α-acid glycoprotein (AGP) [8], GFR [9], Q_R [10], hematocrit [10], kidney weight [10], and relative ontogeny for transporter-mediated intrinsic clearance (ont_T). The functions for ont_T described either hypothetical values, or published functions for individual [3] or aggregated [11,12] transporter systems.

The concentrations of the two plasma proteins impact the f_u of the drug in plasma and the hematocrit levels impact BP. $CL_{int,sec}$ was obtained as the product of transporter-mediated intrinsic clearance ($CL_{int,T}$), ont_T, the number of proximal tubule cells per gram kidney (PTCPGK), and kidney weight (KW), as shown in equation [2].

$$CL_{int,sec} = CL_{int,T} \times ont_T \times PTCPGK \times KW \quad [2]$$

$CL_{int,T}$ is the resultant of expression and activity of renal secretion transporters. While maturation functions for KW and ont_T were included in the pediatric PBPK model for CL_R, the number of proximal tubule cells per gram kidney was assumed to have the same value in children as in adults (60 × 106 cells), as no information was available about its development. KW (g) was calculated across the pediatric age by multiplying the kidney volume (L) with a kidney density of 1050 g/L as obtained from Simcyp v18. All maturation functions and parameter values on which the PBPK model for CL_R is dependent, can be found in Table 5.1. These maturation functions are depicted in Figure 5.1A.

Ont_T was included in equation [2] as a fraction relative to the adult $CL_{int,T}$ In this way, pediatric $CL_{int,T}$: (1) remained fixed at the adult $CL_{int,T}$ levels (ont_T = 1, meaning ontogeny is absent), (2) was a constant fraction of the adult $CL_{int,T}$ throughout the entire pediatric age-range, or (3) increased with age as flexible fraction of adult $CL_{int,T}$ according to published ontogeny functions [3]. For the relative ontogeny fractions

that remained constant throughout the pediatric age, the following values were used: 0.05, 0.2, 0.5, 0.7. Ontogeny functions that increased with age were taken from literature, including 4 functions for individual transporters [3] (i.e. OAT1, OAT3, OCT2, and Pgp), and 2 functions for aggregated transporter systems [11,12]. All the relative ontogeny functions for $CL_{int,T}$ that increased with age and the details about their implementation in the model are presented in Table 5.1. In addition, the published ontogeny functions that characterize relative ontogeny for individual (i.e. OAT1, OAT3, OCT2, and Pgp) and aggregated (i.e. Hayton et al., DeWoskin et al.) transporters throughout the pediatric population relative to adult values, are visualized in Figure 5.1B.

The pediatric PBPK-based model was used to predict CL_R in typical virtual individuals. For this, patients with the following ages were selected: 1 day, 1, 3, and 6 months, 1, 2, 5, and 15 years for pediatric

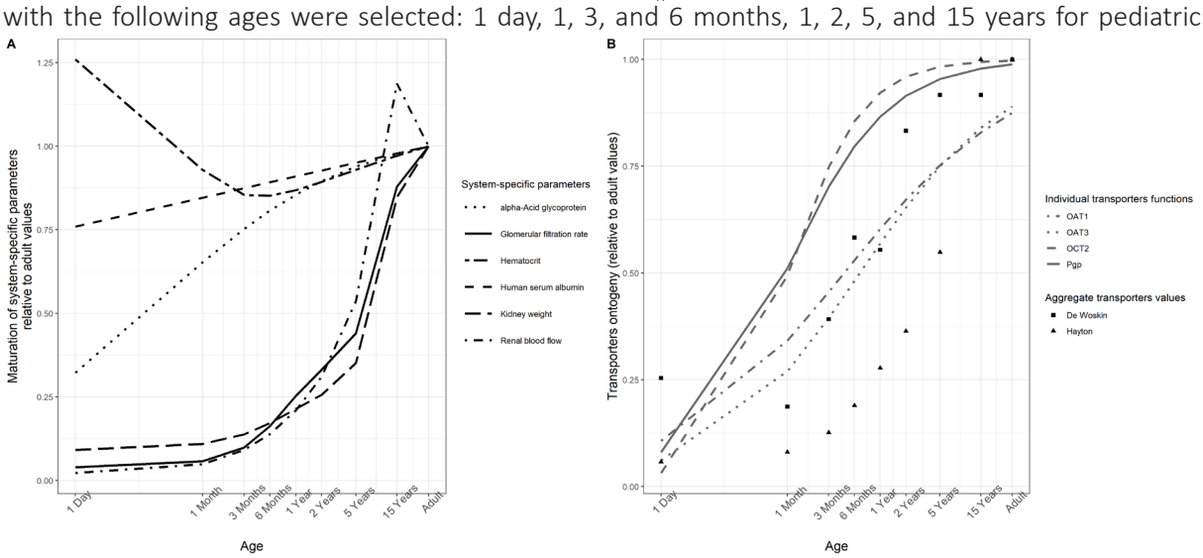

Figure 5.1 – Published functions illustrating (A) the maturation of system-specific parameters and (B) age-dependent ontogeny functions (ontT) for individual or aggregated transporter systems used with the transporter-mediated intrinsic clearance (CLint,T) to obtain intrinsic secretion clearance (CLint,sec). These functions were used to extend the PBPK model to the pediatric population according to the functions in Table 1.

individuals, and 35 years for the adult. The demographics for the typical pediatric individuals required to obtain the maturation functions in the PBPK-based model were derived from the NHANES database [13] and the ones for the typical adult were derived from the ICRP annals [14]. The demographic characteristics corresponding to these ages are given in Table 5.2.

For a systematic investigation of the drug-specific parameter space, hypothetical drugs with different properties were generated and their CL_R was predicted with the PBPK model for CL_R for all typical individuals. The hypothetical drugs were defined by four drug-specific properties for which ranges of realistic values were used as follows:

- The drugs were assumed to bind exclusively to either HSA or AGP.
- $f_{u,adult}$ values of 0.05, 0.15, 0.25, 0.35, 0.45, 0.55, 0.65, 0.75, 0.85, 0.95 and 1 were used for drugs binding to either HSA or AGP.
- BP was obtained from hematocrit levels and Kp values in adults of 0, 1, 2, 3 and 4 (Table 5.1) [15].
- For $CL_{int,T}$ 39 representative values were sampled within the range of 2 and 500 ml/min/mg protein.

The selected range was based on $CL_{int,T}$ values obtained from published CL_R values in adults following retrograde calculation for 53 drugs that are renally excreted by ATS. The obtained $CL_{int,T}$ represents the affinity of the drug for different transporters together with the abundances of transporters. Details about the retrograde calculation of $CL_{int,T}$ are shown in the Supplement section S5.1: Retrograde-calculation of $CL_{int,T}$ from adult CL_R values and the obtained $CL_{int,T}$ values for these drugs in adults are displayed in Figure S5.1.

Table 5.1 – Maturation functions used in equations [1] and [2] for the extrapolation of system-specific and combined system-specific and drug-specific model parameters in the physiology-based pharmacokinetic (PBPK) model for renal clearance from typical adults to typical pediatric individuals

System-specific parameters for equations [1] and [2] (abbreviation) [units]	**Maturation functions included in the pediatric PBPK model for CL_R**
Glomerular filtration rate (GFR) [ml/min]	$GFR = 112 \times \left(\frac{WT}{70}\right)^{0.63} \times \left(\frac{PMA^{3.3}}{PMA^{3.3} + 55.4^{3.3}}\right)$
Fraction unbound (fu) [-]	$[HSA]_{ped/adult} = 1.1287 \times \ln(AGE) + 33.746$ $[AGP]_{ped/adult} = \frac{0.887 \times AGE^{0.38}}{8.89^{0.38} + AGE^{0.38}}$ $\rightarrow \boldsymbol{f_{u,ped} = \frac{1}{1 + \frac{(1 - f_{u,adult}) \times [P]_{ped}}{[P]_{adult} \times f_{u,adult}}}}$
Renal blood flow (QR) [ml/min]	$CO = BSA \times (110 + 184 \times e^{-0.0378 \times AGE} - e^{-0.24477 \times AGE})$ $fr = \frac{fr_{males} + fr_{females}}{2}$ $fr_{males} = 4.53 + \left(14.63 \times \frac{AGE}{0.1888 + AGE}\right)$ $fr_{females} = 4.53 + \left(13 \times \frac{AGE^{1.15}}{0.188^{1.15} + AGE^{1.15}}\right)$ $\rightarrow \boldsymbol{Q_R = CO \times fr}$
Intrinsic secretion CL (CLint,sec) [mL/min]	$PTCPGK = 60\ (adult\ value)$ $KW = 1050 \times (4.214 \times WT^{0.823} + 4.456 \times WT^{0.795})/1000$ $\rightarrow \boldsymbol{CL_{int,sec} = ont_T \times CL_{int,T} \times PTCPGK \times KW}$
Blood to plasma ratio (BP) [-]	$hemat = \frac{hemat_{male} + hemat_{female}}{2}$ $hemat_{male} = 53 - \left(\left(43 \times \frac{AGE^{1.12}}{0.05^{1.12} + AGE^{1.12}}\right) \times \left(1 + \left(-0.93 \times \frac{AGE^{0.25}}{0.10^{0.25} + AGE^{0.25}}\right)\right)\right)$ $hemat_{female} = 53 - \left(\left(37.4 \times \frac{AGE^{1.12}}{0.05^{1.12} + AGE^{1.12}}\right) \times \left(1 + \left(-0.80 \times \frac{AGE^{0.25}}{0.10^{0.25} + AGE^{0.25}}\right)\right)\right)$ $\rightarrow \boldsymbol{BP = 1 + hemat \times (f_u \times k_p - 1)}$
Published ontogeny functions for renal transporters (ontT) [-]	$ont_{P-gp} = \frac{PNA^{0.73}}{PNA^{0.73} + 4.02^{0.73}}$ $ont_{OAT_1} = \frac{PNA^{0.43}}{PNA^{0.43} + 19.71^{0.43}}$ $ont_{OAT_3} = \frac{PNA^{0.51}}{PNA^{0.51} + 30.70^{0.51}}$ $ont_{OCT_2} = \frac{PNA^{1}}{PNA^{1} + 4.38^{0.51}}$ $*\ ont_{ATS_{Hayton}} = \frac{(1.08 \times weight^{1.04} \times e^{-0.185 \times age} + 1.83 \times weight^{1.04} \times (1 - e^{-0.185 \times age}))}{ont_{ATS_{Hayton}}(adult)}$ $*\ ont_{ATS_{DeWoskin}} = \frac{20.3}{79.8}, \frac{14.9}{79.8}, \frac{31.3}{79.8}, \frac{46.5}{79.8}, \frac{44.2}{79.8}, \frac{66.5}{79.8}, \frac{73.15}{79.8}, \frac{73.15}{79.8}, \frac{79.8}{79.8}$, *at 1 Day, 1 Month, 3 Months, 6 Months, 1 Year, 2 Years, 5 Years, 15 Years, and Adult, respectively*

WT – bodyweight [kg]
PMA – postmenstrual age [weeks]
HSA – human serum albumin [g/L]
AGP – α-acid glycoprotein [g/L]
[P] – plasma binding protein (e.g. HSA or AGP [g/L])
CO – cardiac output [mL/min]
hemat – hematocrit
fr – fraction of cardiac output directed to renal artery
BSA – body surface area (m2)

AGE – age in [days] for the maturation of [P] and in [years] for the fraction of cardiac output and hematocrit levels
PTCPGK – proximal tubule cells per gram kidney [x 106 cells]
KW – kidney weight [g]
ont_T – transporters ontogeny relative to adult levels [-]
$CL_{int,T}$ – transporter-mediated active clearance [ml/min]
kp – blood-to-plasma partitioning coefficient of a drug
PNA-postnatal age [weeks]

*Hayton et al. developed a continuous function using age in years and weight in kg, based on the data published by Rubin et al. [17]. The function covers the pediatric age-range up to 12 years and values obtained at 12 years were considered mature and assigned to the typical 15-year-old and adult ($ont_{ATS\text{-}Hayton(adult)}$).
*DeWoskin et al. collected literature data on tubular secretion rates and categorized it in different age groups, from neonates up to adults. For children older than 1 year and younger than 18 years, the average between the values published for children and adults was interpolated.

Table 5.2 – Demographics of the typical virtual pediatric individuals13 and adult14 included in this analysis.

Age	Height	Weight	Hematocrit	Body Surface Area
	(cm)	(kg)	(%)	(m2)
1 Day	49.75	3.5	56	0.22
1 Month	54.25	4.3	44	0.25
3 Months	60	5.75	35.5	0.31
6 Months	66	7.55	36	0.37
1 Year	74.75	9.9	36	0.46
2 Years	86	12.35	36.5	0.54
5 Years	108.25	18.25	37	0.73
15 Years	166	54.25	42	1.59
Adult	169.5	66.5	44	1.76

Generating all possible combinations between the values given to the four drug properties yielded 3800 hypothetical drugs that were included in the current systematic analysis.

5.3.2 Contribution of GF and ATS to pediatric CL_R for drugs with different properties

The PBPK-framework was used to simulate CL_R for the 3800 hypothetical drugs for each typical virtual individual. Simulations with a relative ontogeny fixed at adult levels (ont_T = 1) were used to assess the impact of drug-specific properties on CL_R in the absence of transporter ontogeny. For each drug, the relative contribution of GFR and ATS to CL_R was determined according to equations [3a] and [3b], respectively.

$$GFR_{contribution}\% = \frac{CL_{GFR}}{CL_R} \times 100 \quad [3a]$$

$$ATS_{contribution}\% = \frac{CL_{ATS}}{CL_R} \times 100 \quad [3b]$$

5.3.3 Influence of renal transporters ontogeny on pediatric CL_R

To assess the influence of ontogeny of kidney transporters on pediatric CL_R we implemented transporter ontogeny fractions relative to adult values in the pediatric PBPK model for CL_R (equations [1] and [2]) such that ontogeny of $CL_{int,T}$: (1) remained fixed at adult levels, (2) was a constant fraction of adult values throughout the pediatric age-range, or (3) increased with age as a flexible fraction of adult values. The use of these implementations to describe the ontogeny of transporters, enabled us to explore different values and patterns for transporter ontogeny to ultimately quantify the impact of these changes on ATS and CL_R throughout the pediatric age-range. To quantify the influence of transporter ontogeny on pediatric CL_R predictions, a percentage prediction difference (%PD) was calculated between CL_R predictions without ontogeny ($CL_{R\ adult,ont,T}$) (i.e. ontT = 1) and CL_R predictions with transporter ontogeny that either remained constant or increased with age ($CL_{R\ pediatric,\ ont,T}$) according to equation [4].

$$\%PD = \frac{CL_{R_{adult,ont_T}} - CL_{R\ pediatric,ont_T}}{CL_{R_{pediatric,ont_T}}} \times 100 \quad [4]$$

The %PD obtained upon ignoring the ontogeny of kidney transporters was classified as leading to acceptable CL_R predictions for %PD below 30%, reasonably acceptable CLR predictions for %PD between 30% and 50%, and unacceptable CL_R predictions for %PD above 50%. As published transporters ontogeny patterns only increase with age (i.e. ont_T is always between 0 and 1) until they reach adult $CL_{int,T}$ levels (i.e. ont_T = 1), the %PD will always be positive.

In addition, %PD was used to assess the systematic accuracy of CL_R predictions obtained while ignoring transporter ontogeny. CL_R at a certain age would have systematically acceptable predictions for a transporter pathway when the maximum %PD value for all 3800 hypothetical drugs at that pediatric age was below 30%. In this case, ontogeny of transporters was expected to have a limited role in predicting CL_R for any drug at that age. When CL_R predictions obtained in the absence of transporter ontogeny were reasonably acceptable or unacceptable for one or more hypothetical drugs, CL_R predictions were no longer considered systematically acceptable. In this case CL_R predictions might still be acceptable for some of the hypothetical drugs however it cannot be known a priori whether CL_R predictions are acceptable or not for individual drugs, without taking drug properties into account. As such, systematically acceptable scenarios were a means to identify the pediatric ages for which the ontogeny of individual or aggregated transporters cannot be ignored, as it could lead to biased CL_R predictions.

5.4 Results

5.4.1 Contribution of GF and ATS to pediatric CL_R for drugs with different properties

The contributions of GF and ATS to CL_R over age is shown in Figure 5.2 for a selection of 9 hypothetical drugs with varying $CL_{int,T}$ and $f_{u,adult}$ values. These drugs represent the mean and the extremes of the assessed ranges for these parameter values. Here ont_T was fixed at 1, meaning that results show the influence of maturation of system-specific parameters other than transporter ontogeny on CL_R. Very similar results were obtained for drugs binding to AGP (Figure S5.2).

Figure 5.2 and S5.2 show that GF and ATS increase nonlinearly throughout the pediatric age-range with the steepest increase in the first year of life and continue to increase moderately up to the age of 15 years. Clearance by GF is strictly dependent on the maturation of GFR and on the concentrations of drug binding plasma proteins, which impact f_u. Clearance by ATS changes with age and it depends on the maturation of Q_R, KW, concentrations of drug binding plasma proteins, and hematocrit levels, the latter of which impact BP (Table 5.1).

The relative contribution of GF and ATS to CL_R is strongly impacted by $CL_{int,T}$. For drugs mainly cleared by GF (e.g. $CL_{int,T}$ = 5 µL/min/mg protein), the relative contribution of ATS to CL_R is on average 41% and it decreases with age from 52% in neonates to 35% between ages 2 to 15 years. As $CL_{int,T}$ increases, ATS becomes the main pathway for CL_R A 10-fold increase in $CL_{int,T}$ from 5 to 50 µL/min/mg protein increases the relative contribution of ATS, on average, from 41% to 80%. When $CL_{int,T}$ is further increased up to 500 µL/min/mg protein, ATS relative contribution increases up to 90%.

Changes in CL_R are dependent on age-related changes in system-specific parameters as well as on differences in drug-specific parameters. Drugs mainly cleared by GF (e.g. $CL_{int,T}$ = 5 µL/min/mg protein) show, on average, a 15-fold increase in CL_R (from 3 ml/min to 46 ml/min) with $f_{u,adult}$ increasing from 0.05 to 0.95. For drugs mainly cleared by ATS with a $CL_{int,T}$ of 50 µL/min/mg protein, the same increase in $f_{u,adult}$ yields, on average, a 12-fold increase in CL_R (from 11 ml/min to 130 ml/min). For drugs that are mainly cleared by ATS and are largely unbound from plasma proteins ($f_{u,adult}$ = 0.95), a 10-fold increase in $CL_{int,T}$ (from 5 to 50 µL/min/mg protein) yields, on average, a 2.8-fold increase in CL_R (from 46 ml/min to 130 ml/min). For drugs with very high $CL_{int,T}$ values, the same fold-difference in $CL_{int,T}$ (from 50 to 500 µL/min/mg protein) yields, on average, a lower increase in CL_R of only 1.8-fold (from 130 ml/min to 238 ml/min).

Changes in Kp (and implicitly in BP) may only become moderately relevant for drugs with very large $CL_{int,T}$ values and medium to high $f_{u,adult}$ values. When Kp increases from 1 to 4, CL_R increased, on average, only by 1.15 fold for drugs with $CL_{int,T}$ = 50 µL/min/mg protein and $f_{u,adult}$ = 0.55 and reached a maximum increase of 1.25-fold for drugs with $CL_{int,T}$ = 500 µL/min/mg protein and $f_{u,adult}$ = 0.95.

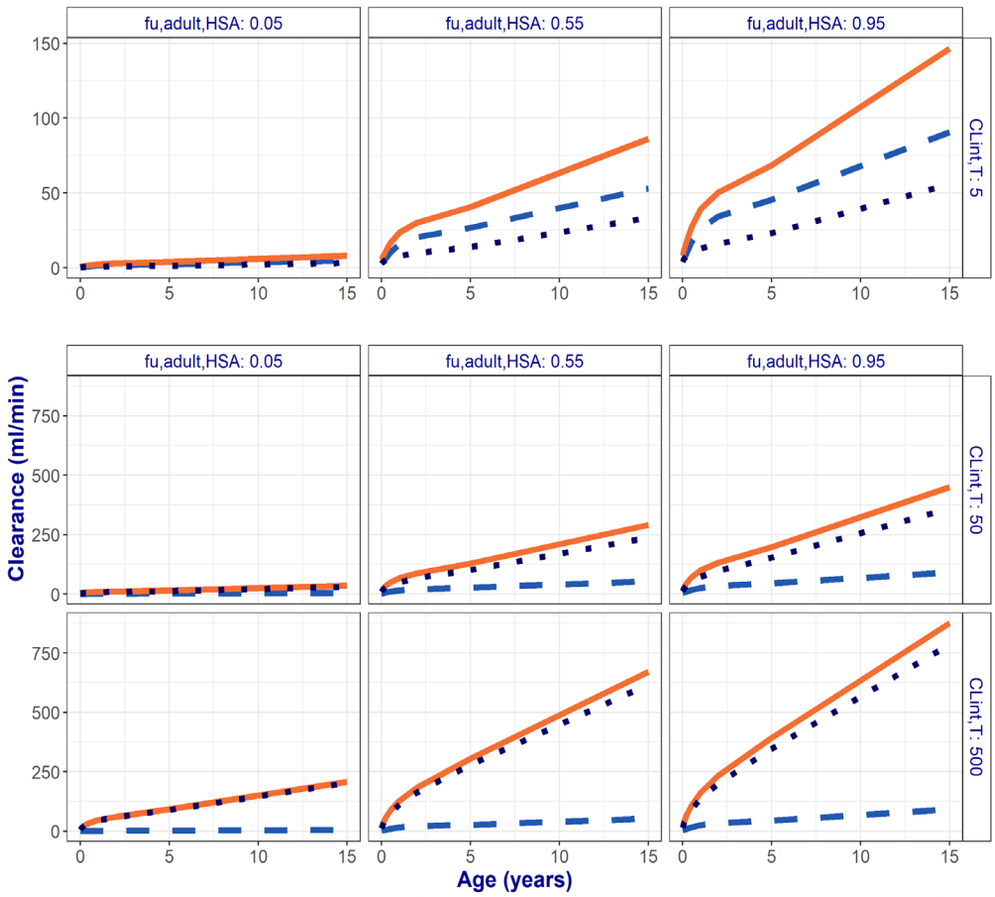

Figure 5.2 – Developmental changes in total renal clearance (CL_R – solid orange lines) and the contribution of glomerular filtration (GF – light blue dashed lines) and active tubular secretion (dark blue dotted lines) vs. age for 9 representative hypothetical drugs. These drugs bind to albumin (HSA) and have low, medium or high unbound fractions in adults ($f_{u,adult}$ - horizontal panels) that change with age, dependent on the HSA plasma concentrations. Transporter-mediated intrinsic clearance values ($CL_{int,T}$) were assumed to remain constant with age at the indicated values (vertical panels).Note the different scales on the y-axes for the graphs in the top row (range 0-150 ml/min) compared to middle and bottom row (range 0-750 ml/min).

5.4.2 Influence of renal transporters ontogeny on CL_R

The role of transporter ontogeny on CL_R was quantified by calculating the %PD between CL_R predictions with the transporter relative ontogeny fixed at adult levels ($CL_{R\ adult,ontT}$ = 1) and CL_R predictions with relative transporter ontogeny that either remains at a constant fraction of adult values or increases over age for individual transporters, as published for OAT1, OAT3, OCT2, Pgp [3], and aggregated transporters [11,12] ($CL_{R\ pediatric,ontT}$).

Figure 5.3 shows the results for the same 9 hypothetical drugs as in Figure 5.2, with four age-constant ontogeny fractions for the renal transporters (i.e. ont_T = 0.05, 0.2, 0.5, 0.7). Similar results are observed for drugs binding to AGP (Figure S5.3). When transporters are underdeveloped (ontT < 0.2), ontogeny of renal transporters cannot be ignored as it would lead to unacceptable CL_R predictions for all investigated hypothetical drugs regardless of age. The shapes of the %PD profiles for the 9 selected drugs differ from one another, depending on whether the primary elimination pathway contributing to CL_R is GF or ATS. This is related to the maturation of other system-specific parameters that are underlying GF and ATS.

For drugs that are mainly cleared by GF ($CL_{int,T}$ = 5 µL/min/mg protein), in children younger than 6 months and relative transporter ontogeny lower than 0.2, ignoring ontogeny of kidney transporters would lead to unacceptable CL_R predictions (%PD = 53%- 113%). For children older than 6 months, with relative ontogeny higher than 0.05, reasonably acceptable CL_R predictions are obtained for all drugs mainly cleared by GF.

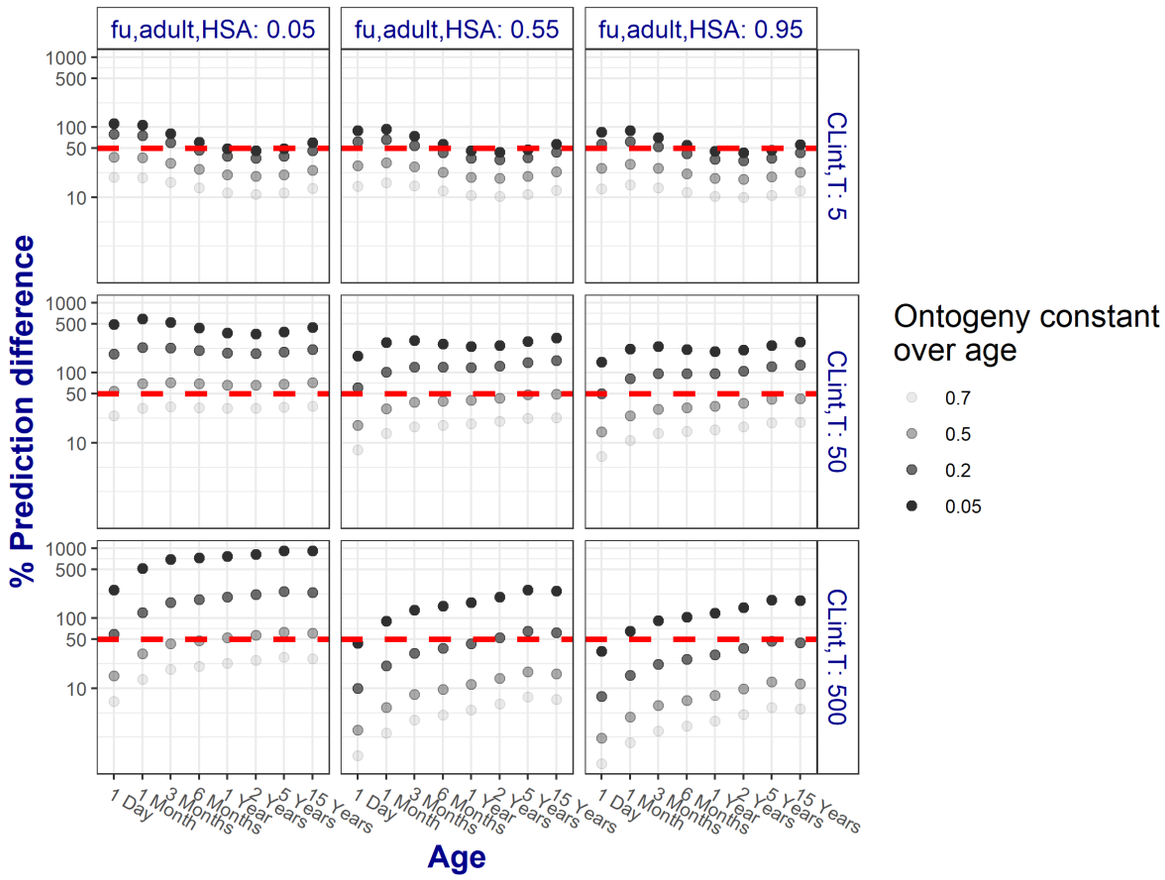

Figure 5.3 – Percentage prediction difference (%PD) for 9 representative hypothetical drugs calculated between renal clearance (CL_R) predictions obtained with the pediatric renal PBPK model that included or excluded hypothetical transporter ontogeny (ont_T) values that remained constant over age. These hypothetical drugs bind to albumin (HSA) and have low, medium or high unbound fractions in adults ($f_{u,adult}$ - horizontal panels) that change with age, dependent on the HSA plasma concentrations. Transporter-mediated intrinsic clearance values ($CL_{int,T}$) were assumed to remain constant with age at the indicated values (vertical panels). The colors of the %PD increases with decreasing transporter ontogeny values (ont_T). The dashed red line represents the threshold of reasonably acceptable CL_R prediction of 50%. Results are displayed on a log-log scale.

For drugs that are mainly cleared by ATS and have a low fraction unbound ($CL_{int,T} \geq 50$ µL/min/mg protein with $f_{u,adult} = 0.05$) ignoring the ontogeny of transporters would lead to unacceptable CL_R predictions (%PD: 53%- 918%) for all pediatric individuals with a low transporter ontogeny ($ont_T \leq 0.5$). For drugs with $CL_{int,T} = 50$ µL/min/mg protein and increasing fu,adult, reasonably acceptable CL_R predictions are obtained for all ages when relative transporter ontogeny is high ($ont_T > 0.5$). For these drugs, %PD can reach values between 50% and 316% when transporter ontogeny is low ($ont_T \leq 0.2$). For drugs with a very large $CL_{int,T}$ and high $f_{u,adult}$ ($CL_{int,T} = 500$ µL/min/mg protein with $f_{u,adult} = 0.95$) the influence of transporter ontogeny on CL_R decreases, as indicated by the reasonably acceptable %PD values.

The results shown in Figure 5.4 complement the previous findings by illustrating the implications for CL_R predictions for drugs that are substrates for transporters for which ontogeny functions have been published. Figure 5.4 shows when CL_R predictions are systematically acceptable with or without transporter ontogeny functions (i.e. CL_R values obtained with ont_T values varying with age according to individual [3] or aggregated [11,12] transporters functions for ontogeny and CL_R values obtained with ont_T fixed to the adult levels ($ont_T = 1$)). In both simulations, system-specific parameters and transporter ontogeny functions changed with age as shown in the Table 5.1 and Figure 5.1.

Figure 5.4 displays the results as a heat-map, where the numbers in each box represent the minimum, median and maximum %PD values obtained for all 3800 hypothetical drugs that are substrates for the indicated individual transporter or aggregated transporters at every pediatric age. Systematically

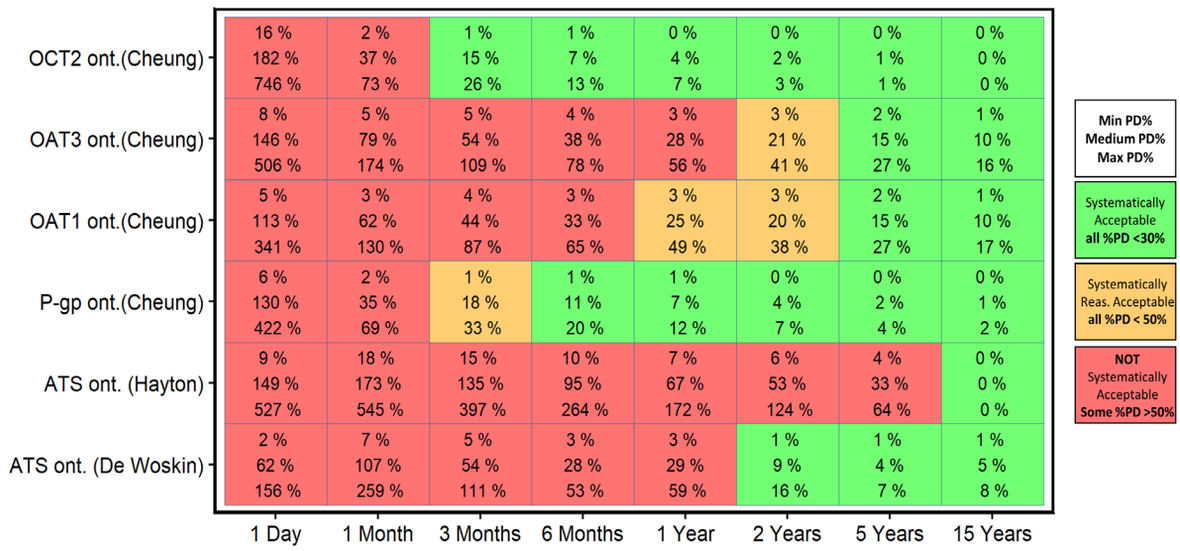

Figure 5.4 –Percentage prediction difference (%PD) between CL_R predictions obtained with the pediatric PBPK model that does not include transporter ontogeny (ont_T = 1, reflecting adult values) and the model that includes age-specific pediatric ont_T values for each of the indicated transporter systems. In each box, the minimum (top), median (middle) and maximum (bottom) %PD is displayed to summarize the findings for all hypothetical drugs per typical pediatric individual at different ages. Systematically acceptable scenarios have %PD for all drugs < 30% (green box), reasonable acceptable scenarios have %PD for all drugs < 50% (orange box), and absence of systematic acceptance means that at least one drug has a %PD > 50% (red box).

acceptable scenarios are achieved when CL_R predictions for all 3800 hypothetical drugs lead to %PD values below 30% in the absence of transporter ontogeny. This is indicated by the green boxes, while orange and red boxes indicate CL_R predictions that are reasonably acceptable (highest %PD between 30% and 50%) and unacceptable (highest %PD > 50%), respectively, for one or more drugs. Nonetheless, when CL_R predictions are not systematically acceptable it does not imply that %PD values below 30% were not observed, rather it indicates that predictions for one or more drugs are biased at the indicated age. Hence, it cannot be predicted a priori whether the predictions without including ontogeny of transporters will be acceptable or not, without taking drug properties into account.

When the relative transporter ontogeny varied with age according to the functions of Cheung et al. (i.e. for OAT1, OAT3, OCT2, and P-gp) [3], ignoring ontogeny lead to CL_R predictions that were not systematically acceptable for all transporters in newborns of 1 month and younger. CL_R predictions of drugs that are substrates of OAT transporters are not systematically acceptable below the age of 1 year. For children of 2 years and older ignoring the ontogeny of transporters lead to CL_R predictions that were reasonably acceptable or acceptable for all transporters – individual or aggregated- and all substrates, except when ontogeny follows the aggregated transporters ontogeny function as published by Hayton et al..

5.5 Discussion

A PBPK-based framework was used to predict CL_R of hypothetical drugs with various properties that are substrates for renal secretion transporters throughout the pediatric age-range. This approach provided insight on the contribution of GF and ATS to total pediatric CL_R. In addition, the impact of ignoring this transporter ontogeny in predicting CL_R in children was quantified.

The physiology-based model for CL_R used in the presented framework was developed based on a model published for adults [5] that was extended to the pediatric population by including maturation functions for the system-specific parameters as shown in Table 5.1 and illustrated in Figure 5.1A. This model included two major contributing pathways to CL_R: GF and ATS. Based on this model we could quantify the impact of transporter ontogeny on pediatric drug clearance for all current and future small molecule

drugs, based on drug-specific properties alone. We found that the contribution of these pathways to CL_R increases non-linearly throughout the pediatric age-range, with the steepest increase during the first year of life, even in the absence of transporter ontogeny. These changes in pediatric CL_R are determined by the influence of maturation in the system-specific parameters underlying GF and ATS as well as by drug-specific properties (Figure 5.2). Both GF and ATS increase with increasing f_u, while ATS also increases with increasing $CL_{int,T}$ values.

Drug f_u was found to have a major influence on CL_R through both investigated pathways, but especially on CL_R through GF. $CL_{int,T}$ has a major influence on CL_R only through ATS. Drugs with 10-fold different $CL_{int,T}$ values and low binding to plasma proteins ($f_{u,adult}$ = 0.95) yield different contributions of ATS to CL_R. When ATS contribution to CL_R is limited only by the activity and the abundance of transporters (i.e. $CL_{int,T}$ changes between 5 and 50 µL/min/mg protein) an increase of 1.9-fold in average ATS contribution was observed. As $CL_{int,T}$ changes between 50 and 500 µL/min/mg protein) we observed a lower increase in average ATS contribution of only 1.1-fold16. This behavior could be explained by the fact that f_u and $CL_{int,sec}$ are rate limiting factors for ATS when $CL_{int,sec}$ x f_u is low relative to Q_R (i.e. permeability limited process). Q_R becomes the rate limiting factor for ATS when $CL_{int,sec}$ x f_u is high relative to Q_R (i.e. perfusion limited process). This also explains why the impact of ignoring transporter ontogeny decreases for drugs with very high $CL_{int,T}$, as shown by the lower %PD values in Figure 5.3. It is important to mention that whether ATS is permeability limited ($CL_R/Q_R < 0.3$) or perfusion limited ($CL_R/Q_R > 0.7$) or a combination between the two processes ($0.3 < CL_R/Q_R < 0.7$) may change with age, as shown in Figure 5.5.

The present framework explored a broad parameter space for ontogeny of transporters. By keeping ontogeny of transporters constant with age, the potential impact of ignoring ontogeny on predicting CL_R was systematically explored (Figure 5.3). This exploration highlights that an ontogeny below 0.2 of the adult value cannot be ignored for the majority of drugs regardless of the pediatric age. In this situation, the assumption that there are no differences in transporter ontogeny between children and adults would lead to unacceptable CL_R predictions. Data characterizing how ontogeny of individual kidney transporters changes across the pediatric age is scarce in literature. Cheung et al. [3] recently took the first steps in quantifying the ontogeny of protein abundance for individual renal transporters. According to this report, which is based on a limited sample size, BCRP, MATE1, MATE2-K, and GLUT2 have protein abundance levels similar to the adult levels throughout the studied pediatric age-range [3], meaning that ont_T = 1 for children of all ages and that transporter ontogeny is not a factor of influence in predicting CL_R for substrates of these transporters. Including these ontogeny profiles in the current framework increased our understanding on the role of age-dependent ontogeny in predicting CL_R (Figure 5.4). As reported by Cheung et al., the ontogeny of OAT1 and OAT3 is slower than the ontogeny of OCT2 and P-gp. Ignoring OCT2 ontogeny yields systematically acceptable pediatric CL_R values for all its hypothetical substrates in children from 3 months and older. For P-gp substrates, the same holds true in children from 6 months and older. Ontogeny of OATs however cannot be ignored for children younger than 2 years as CL_R predictions are not systematically acceptable for substrates of this transporter. The CL_R predictions obtained with the aggregate transporter function published by DeWoskin et al. [11] are in line with the results for OATs. The aggregate function of Hayton et al. [12] suggest a much slower ontogeny leading to CL_R predictions that are not systematically acceptable in children up to and including 5 years. CL_R predictions with Hayton et al. [12] diverge from the predictions obtained with the other transporter ontogeny functions since it was the first function to quantify the ontogeny of ATS and has a different profile than all the other studied functions. Disregarding ontogeny of transporters leads to over-predictions of CL_R in the young patients. if these predicted CL_R values were used as the basis for pediatric dose adjustments, these could lead to over-exposure to drugs and, eventually, increase the risk of toxic events.

As our analysis identifies drugs for which CL_R is sensitive to transporter ontogeny, the proposed framework can also be used to find and select drugs with relevant properties to serve as *in vivo* probes

for the quantification of the ontogeny of transporters underlying ATS. From the results of the current analysis we could conclude that the best probe drugs should have a $CL_{int,T}$ of 5-50 µL/min/mg protein and medium to high fraction unbound in adults ($f_{u,adults}$ = 0.55 – 0.95). Drugs for which GF is the main elimination pathway or drugs with extremely high $CL_{int,T}$ that cause renal blood flow to be limiting for elimination, will have a limited use in characterizing ontogeny profiles. These guidelines could be the basis for future research aiming to derive ontogeny of individual renal transporters *in vivo*.

Our results rely on the validity of the PBPK approach, which is currently considered the "gold standard" for clearance predictions in the absence of clinical data. This approach gives an overview of the impact of system- and drug-specific parameters on CL_R. The explored arrays of ontogeny fractions and of drug properties were realistic, however, unrealistic combinations of drug properties could have been generated. As with the previously published hepatic PBPK framework [6], this analysis does not include measures for the variability or uncertainty of the parameters that constitute the PBPK model, to highlight the impact of system- and drug-specific changes in the absence of variability and uncertainty. Our approach could be extended for investigations on the impact of variability and uncertainty by including variability terms on the system-specific parameters and performing stochastic simulations. Finally, PBPK modelling is ideally suitable to study the impact of specific physiological processes in a way that is not possible *in vivo*. In the *in vivo* situation, studies are limited to drugs that are currently available on the market and prescribed to children. However, generally these drugs are not eliminated in totality by one single pathway. Moreover, the accuracy of these observations is impacted by aspects related to study design, sampling and analytical methods. Our current model-based analysis is not impacted by these limitations. The physiology-based model for CL_R used here only included GF and ATS, but not passive permeability, reabsorption, or renal metabolism. This enabled the study of GF and ATS in isolation and reduced the noise and complexity of the results. The influence of ontogeny on transporters working in tandem or of reabsorption and kidney metabolism together with their dependencies on physiological

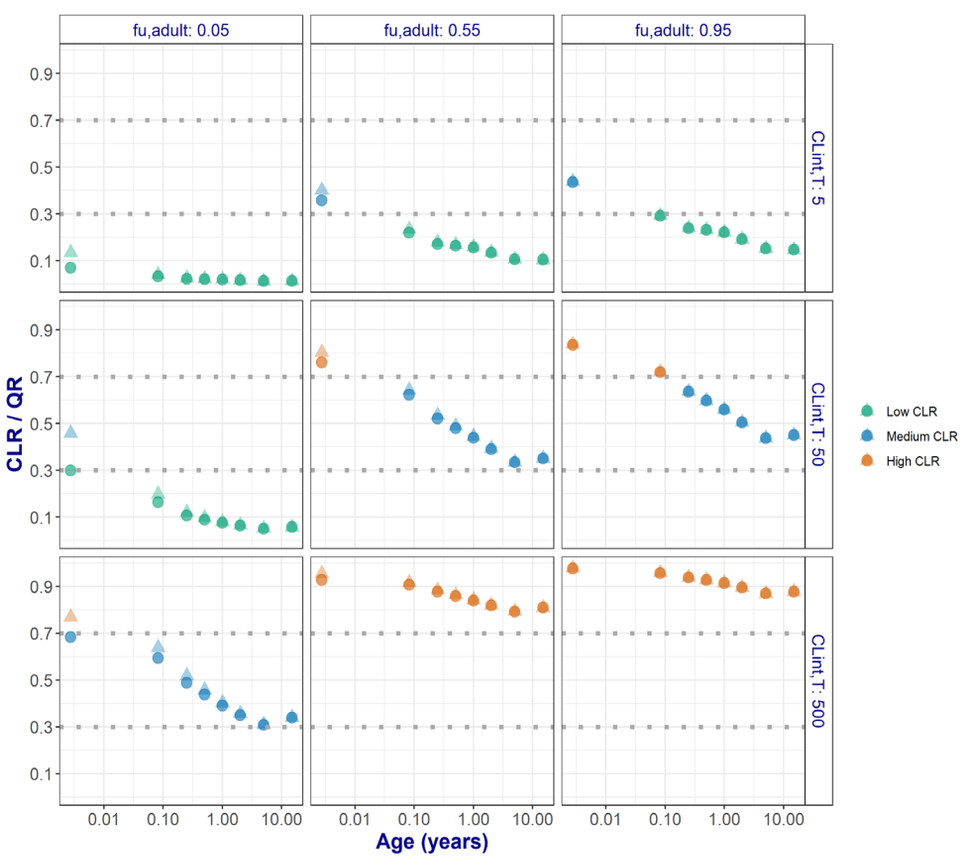

Figure 5.5 – Ratio of total renal clearance (CLR) and renal blood flow (Q) for 9 representative hypothetical drugs. Results are presented for drugs binding to human serum albumin (HSA) (circles) or to α-acid glycoprotein (AGP) (faded triangles).

properties like pH at the tubule side, ionization, enzyme abundance, affinity, and maturation, could be explored in a similar manner in subsequent analyses.

5.6 Conclusion

A PBPK-based framework was used to determine the role of drug properties and ontogeny of transporters in predicting pediatric CL_R. The contribution of GFR to CL_R is influenced by drug f_u and contribution of ATS to CL_R is influenced by f_u and $CL_{int,T}$. Transporters play a major role in predicting CL_R. Discordance in the CL_R predictions when ignoring maturation in ATS, shows when accurate predictions of total pediatric CL_R from the adults if extrapolation solely relied on changes in GF with age, are not possible. Ignoring transporter ontogeny, especially when it is below 0.2 of the adult values, leads to inaccurate CL_R predictions for the majority of drugs, regardless of age. Given known age-dependent patterns, transporter ontogeny cannot be ignored in children younger than 2 years. Drugs with properties that lead to high %PE when ignoring ATS ontogeny may serve as sensitive *in vivo* probes to further investigate transporter ontogeny.

5.7 Acknowledgements

The authors would like to thank Muhammed Saleh for reviewing the R code used for this work.

5.8 References

1. Morrissey, K. M., Stocker, S. L., Wittwer, M. B., Xu, L. & Giacomini, K. M. Renal transporters in drug development. Annu Rev Pharmacol Toxicol. 503–29 (2013). doi:10.1146/annurev-pharmtox-011112-140317
2. Kunze, A., Huwyler, J., Poller, B., Gutmann, H. & Camenisch, G. In vitro-in vivo extrapolation method to predict human renal clearance of drugs. J. Pharm. Sci. (2014). doi:10.1002/jps.23851
3. Cheung, K. W. K. et al. A Comprehensive Analysis of Ontogeny of Renal Drug Transporters: mRNA Analyses, Quantitative Proteomics, and Localization. Clin. Pharmacol. Ther. (2019). doi:10.1002/cpt.1516
4. Elmorsi, Y., Barber, J. & Rostami-Hodjegan, A. Ontogeny of hepatic drug transporters and relevance to drugs used in pediatrics. Drug Metabolism and Disposition (2016). doi:10.1124/dmd.115.067801
5. Rowland Yeo, K., Aarabi, M., Jamei, M. & Rostami-Hodjegan, a. Modeling and predicting drug pharmacokinetics in patients with renal impairment. Expert Rev Clin Pharmacol 4, 261–274 (2011).
6. Calvier, E. et al. Allometric scaling of clearance in paediatrics: when does the magic of 0.75 fade? Clin Pharmacokinet. 56, 273–285 (2017).
7. Krekels, E. H. J., Calvier, E. A. M., van der Graaf, P. H. & Knibbe, C. A. J. Children Are Not Small Adults, but Can We Treat Them As Such? CPT Pharmacometrics Syst. Pharmacol. (2019). doi:10.1002/psp4.12366
8. Johnson, T. N., Rostami-Hodjegan, A. & Tucker, G. T. Prediction of the clearance of eleven drugs and associated variability in neonates, infants and children. Clin. Pharmacokinet. 45, 931–956 (2006).
9. Salem, F., Johnson, T. N., Abduljalil, K., Tucker, G. T. & Rostami-Hodjegan, A. A re-evaluation and validation of ontogeny functions for cytochrome P450 1A2 and 3A4 based on in vivo data. Clin. Pharmacokinet. 53, 625–636 (2014).
10. Simcyp (a Certara Company). Simcyp v18. (2018).
11. DeWoskin, R. S. & Thompson, C. M. Renal clearance parameters for PBPK model analysis of early lifestage differences in the disposition of environmental toxicants. Regul. Toxicol. Pharmacol. 51, 66–86 (2008).
12. Hayton, W. L. Maturation and growth of renal function: dosing renally cleared drugs in children. AAPS PharmSci 2, E3 (2000).
13. National Health and Nutrition Examination Survey. www.cdc.gov/growthcharts/index.htm.
14. International Commission on Radiological Protection. Basic anatomical and physiological data for

use in radiological protection- Skeleton. Ann. ICRP 32, 1–277 (1995).

15. Rowland, M. & Tozer, T. N. Clinical Pharmacokinetics and Pharmacodynamics: Concepts and Applications. (Wolters Kluwer Health/Lippincott William & Wilkins, 2011).
16. Tucker, G. Measurement of the renal clearance of drugs. Br. J. Clin. Pharmacol. (1981). doi:10.1111/j.1365-2125.1981.tb01304.x

5.9 Supplementary material

S5.1: Retrograde calculation of transporter-mediated intrinsic clearance from adult renal clearance values

Following an extensive literature search, Scotcher et al. [1] published data on renal clearance (CL_R) of 157 drugs in adults. These drugs were classified according to the publication of Varma et al. [2] into (i) compounds with net renal reabsorption (CL_R < 0.8 x fu x GFR), (ii) compounds with net renal secretion (CL_R > 1.2 x fu x GFR) and (iii) compounds with no net reabsorption or secretion (0.8 x fu x GFR < CL_R < 1.2 x fu x GFR). Only findings on the 53 net secretion drugs were used in this analysis [2].

By solving equation [S1] for $CL_{int,sec}$ we obtain [S1A], where all terms are known and all parameter values take adult values.

$$CL_R = f_u \times GFR + \frac{(Q_R - GFR) \times f_u \times CL_{int,sec}}{Q_R + f_u \times \frac{CL_{int,sec}}{BP}} \quad [S1]$$

$$CL_{int,sec} = \frac{(CL_R - f_u \times GFR) \times Q_R}{((Q_R - GFR) \times f_u \; - (CL_R - f_u \times GFR) \times \frac{fu}{BP})} \quad [S1A]$$

To get $CL_{int,T}$ we solved equation [S2] for $CL_{int,T}$ and obtained the form in [S2A], where all parameter values take adult values and $CL_{int,sec}$ from equation [S1A] is used in equation [S7A].

$$CL_{int,sec} = ont_T \times CL_{int,T} \times PTCPGK \times KW \quad [S2]$$

$$CL_{int,T} = \frac{CL_{int,sec}}{ont_T \times PTCPGK \times KW} \quad [S2A]$$

The $CL_{int,T}$ values obtained for 53 drugs classified as net secretion drugs following the retrograde calculation are shown in Figure S5.1.

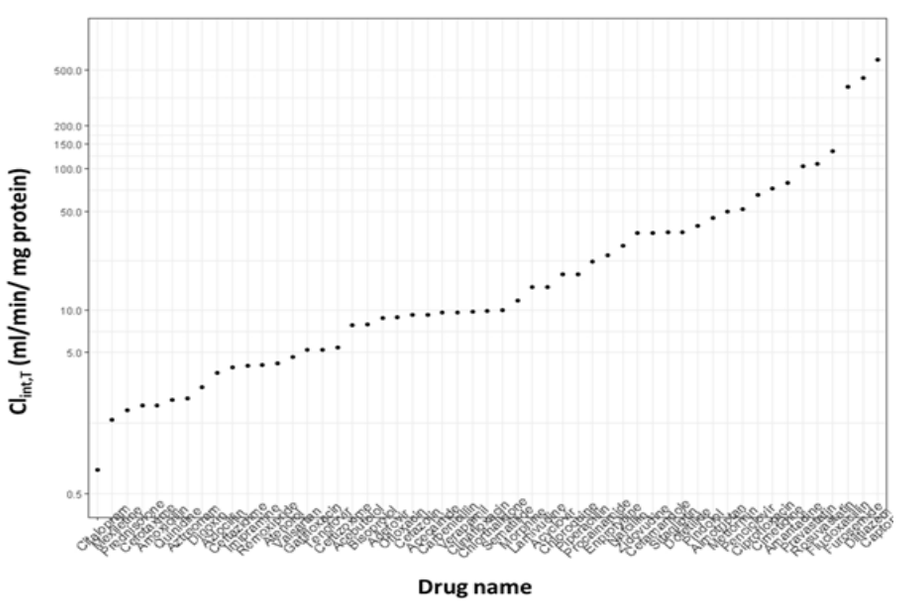

Figure S5.1 – Intrinsic clearance ($CL_{int,T}$) values obtained for 53 drugs classified as net secretion drugs collected from literature. Drugs are ordered by $CL_{int,T}$ values. Y-axis is logarithmic.

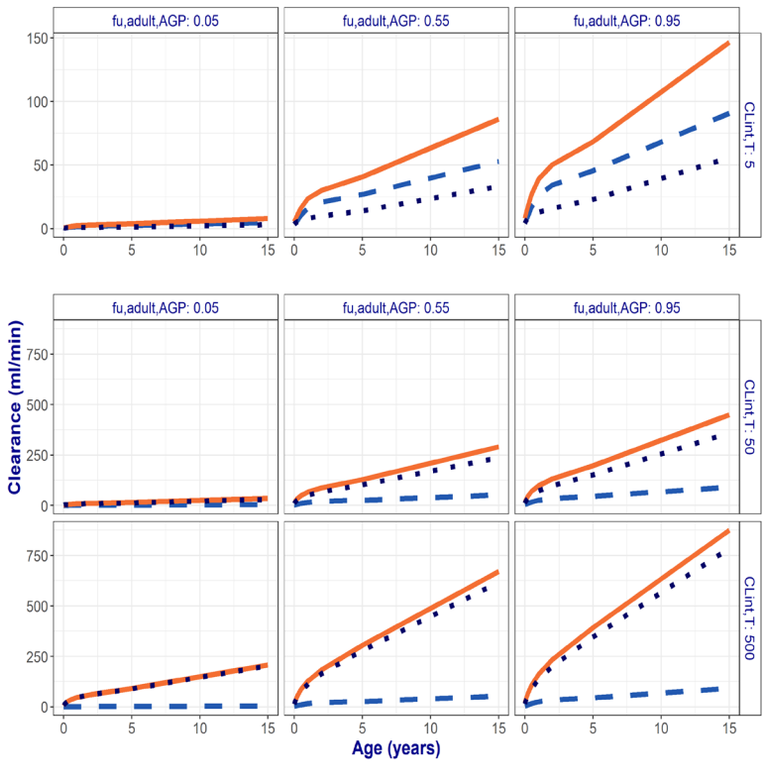

Figure S5.2 – Developmental changes in total renal clearance (CL_R – solid orange lines) and the contribution of glomerular filtration (GF – light blue dashed lines) and active tubular secretion (dark blue dotted lines) vs. age for 9 representative hypothetical drugs. These drugs bind to α-acid glycoprotein (AGP) and have low, medium or high unbound fractions in adults ($f_{u,adult}$ - horizontal panels) that change with age, dependent on the AGP plasma concentrations. Transporter-mediated intrinsic clearance values ($CL_{int,T}$) were assumed to remain constant with age at the indicated values (vertical panels)..Note the different scales on the y-axes for the graphs in the top row (range 0-150 ml/min) compared to middle and bottom row (range 0-750 ml/min).

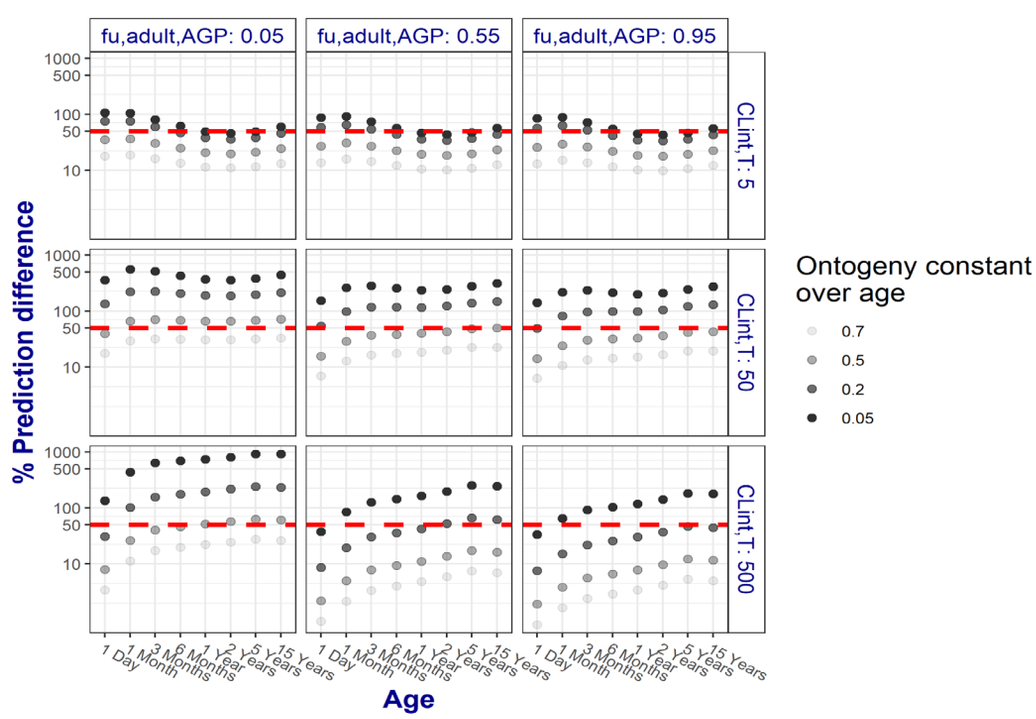

Figure S5.3 – Percentage Prediction difference (%PD) for 9 representative hypothetical drugs calculated between renal clearance (CL_R) predictions obtained with the renal PBPK model that included or excluded hypothetical transporter ontogeny (ont_T) values that remained constant over age. These drugs bind to α-acid glycoprotein (AGP) and have low, medium or high unbound fractions in adults ($f_{u,adult}$ - horizontal panels) that change with age, dependent on the AGP plasma concentrations. Transporter-mediated intrinsic clearance values ($CL_{int,T}$) were assumed to remain constant with age at the indicated values (vertical panels). The colors of the %PD increases with decreasing transporter ontogeny values (ont_T). The dashed red line represents the threshold of reasonably acceptable CL_R prediction of 50%. Results are displayed on a log-log scale.

5.10 References Supplementary material

1. Scotcher D, Jones C, Rostami-Hodjegan A, Galetin A (2016) Novel minimal physiologically-based model for the prediction of passive tubular reabsorption and renal excretion clearance. Eur J Pharm Sci. https://doi.org/10.1016/j.ejps.2016.03.018
2. Varma MVS, Feng B, Obach RS, Troutman MD, Chupka J, Miller HR, El-Kattan A (2009) Physicochemical determinants of human renal clearance. J Med Chem 52:4844–4852 . https://doi.org/10.1021/jm900403j

5.11 R code

```
# Script: PBPK Framework - Renal
# Version: 12
# Last Update: 6-2-2020
# Author: SC
#==============================
# change with previous version: Removed interim plots;
#
# Create the dataframe with all required combinations
#============================================================
# remove all from environment:
rm(list=ls(all=TRUE))
# change for parth

memory.limit(200*1024*1024*1024)
# Load Libraries
library(dplyr)    # operations with dataframes
library(ggplot2)  # plotting
library(gridExtra)# multiplot
library(reshape2) # melt and cast
library(cowplot)  # extra options for ggplot plots
#=====================

# Input the wd (change home location and wd as needed):

home <-""
wd <- "1.Scripts\\"

# Call script with functions that are needed:
# ---

source(file = paste(home, wd, "SC04_PBPKFrame_functions.R", sep =""))

# Generate demographics dataframe:
demo <- data.frame(
  lab = c("1 Day","1 Month", "3 Months", "6 Months", "9 Months", "1 Year",
"2 Years", "5 Years", "10 Years", "15 Years", "Adult"), #labels for ploting
  age = c(1/365,30/365,0.25,0.5,0.75,1,2,5,10,15,35), # in years
  wt = c(3.45,4.3,5.75,7.55,8.9,9.9, 12.35, 18.25, 32.5,54.25, (73+60)/2),
# in kg
  ht = c(49.75,54.25,60,66,70.75,74.75,86,108.25, 138.25,166,(163+176)/2),
# in cm; added to calculate the BSA needed for CO%
  hemat = c(56, 44, 35.5, 36, 36, 36, 36.5, 37, 40, 42, 44) / 100,
      # Hematocrite in percentage for each age (AGE), from Am Fam Physi-
cian. 2001 Oct 15;64(8):1379-86.Anemia in children.Irwin JJ
  mat_w = round(x = c(20.3, 14.9, 31.3, 46.5, 45.3, 44.2, 66.5, 73.15,
73.15, 73.15, 79.8)/79.8,digits = 3) # aggregated ATS ontogney function de-
rived from deWoskin
)

# Generate age specific renal blood flow (qr), glomerular filtration rate
(gfr), kidnew weight (kw in g)
# ---

demo$kw <- KW(demo$wt)
demo$qr <- QR_new(age = demo$age, ht = demo$ht, wt = demo$wt)
demo$gfr <- GFR(wt = demo$wt, age = demo$age)

# Include age-specific functions for ontogeny:
# ---
```

```
# aggregated ontogeny function of Hayton 2000:

  demo$mat_h <- rfp(wt = demo$wt, age = demo$age)/rfp(wt = demo$wt[demo$lab
== "10 Years"], age = demo$age[demo$lab == "10 Years"]) # % of 5yo maximal
capacity (assumption for ages > 15yr is 100%) Hayton 2000 not defined abve
12 year, 35.5 kgs
  demo$mat_h[demo$lab %in% c("15 Years", "Adult")] <- 1 # asume 15 yo and
adult to have 100% maturation since they are out of range.

# individual transporter ontogeny functions:

  demo$mat_pgp <- pgp(demo$age)
  demo$mat_oat1 <- oat1(demo$age)
  demo$mat_oat3 <- oat3(demo$age)
  demo$mat_oct2 <- oct2(demo$age)

# assumed maturation as fraction of adult value (fixed to 1; to use later in
impact of ont heatmap)

  mat <- 1 #c(0.1, 0.25, 0.5, 0.75, 1)

# For manuscript/review purposes remove a few ages:
# ---

  demo <- demo[!demo$lab %in% c( "9 Months", "10 Years"),]

# Vectors with arrays for generating the hypothetical drug
# ---

  fu <- round(seq(0.05,1, by =0.1), digits = 2) # reference/ adult plasma
fraction unbound values
  kp <- c(0, 1, 2, 3, 4) # range from Tozer and Rowland book
  freab <- 0 # fraction reabsorbed


# Used back-calculated clint values from Varma 2009 and D.Scotcher adult
CLR values, class: "Net Secretion"
# to determine realistic range to investigate
# ---
  clint <- c(seq(0, 20, by = 1), seq(30, 70, by = 5), seq(80, 100, by =
10), 150, seq(200, 600, by = 100)) # uL/min

# Expanded dataset with all possible combinations; keep system parameters
unchanged:
# ---

  d <- expand.grid(age = demo$age, fu = fu, kp = kp, freab = freab, clint =
clint, mat = mat)
  d <- left_join(x = demo, y = d, by = "age")

# Add maturation functions for fu dependent on plasma proteins concs.
# ---

  d$fu_paed_hsa <- fu_paed_hsa(age_ad = demo[demo$lab == "Adult", "age"],
age = d$age, fu = d$fu) # HSA
  d$fu_paed_aag <- fu_paed_aag(age_ad = demo[demo$lab == "Adult", "age"],
age = d$age, fu = d$fu) # AGP

# Plots
# ---
  wd <- "2.Figures\\"
```

```
# Figure with ontogeny transporters functions:
# -- log normal data:

  linety <- c("Pgp" = 1, "OCT2" = 2, "OAT3" = 3, "OAT1" = 4)
  sha <- c("De Woskin" = 15, "Hayton" = 17)

tiff(paste0(home, wd,"SC_Figure_S2_TransportersOntog_func.tiff"), width = 22,
height = 10, res = 300, units = "cm")

  ggplot(demo)+
    geom_line(aes(age, mat_pgp, linetype = "Pgp"), size = 1.2, col =
"#68686b")+
    geom_line(aes(age, mat_oct2, linetype = "OCT2"), size = 1.2, col =
"#68686b")+
    geom_line(aes(age, mat_oat3, linetype = "OAT3"), size = 1.2, col =
"#68686b")+
    geom_line(aes(age, mat_oat1, linetype = "OAT1"), size = 1.2, col =
"#68686b")+
    geom_point(aes(age, mat_w, shape = "De Woskin"), size = 1.75, col =
"black")+
    geom_point(aes(age, mat_h, shape = "Hayton"), size = 1.75, col =
"black")+
    scale_x_continuous(breaks = demo$age, labels = demo$lab, trans =
"log10")+
    scale_linetype_manual(name = "Individual transporters functions", val-
ues = linety)+
    scale_shape_manual(name = "Aggregate transporters values", values =
sha)+
    xlab("Age(years)")+
    ylab("Transporters ontogeny (relative to adult values)")+
    theme(axis.title.x = element_text(size = 10),
          axis.title.y = element_text(size = 10),
          axis.text.x = element_text(angle = 15))

dev.off()

# melt dataframe to be able to group by transporter:

d_melt <- melt(d, id.vars = c("lab", "age", "wt", "kw", "ht", "hemat",
"qr", "gfr", "fu", "kp", "freab",
                                "clint", "fu_paed_hsa", "fu_paed_aag"),var-
iable.name = "transp", value.name = "mat_fr")
d <- d_melt
d$mat_flag <- ifelse(d$mat_fr > 0 & d$mat_fr <= 0.25, "0-25%",
                     ifelse(d$mat_fr > 0.25 & d$mat_fr <= 0.5, "25-50%",
                            ifelse(d$mat_fr > 0.5 & d$mat_fr <= 0.75, "50-
75%", "75-100%")))

# PBPK clearance simulations for the pediatric population for each plasma
protein:

d$cl_mat_hsa <- CL(qr = d$qr, wt = d$wt, age = d$age, fu = d$fu_paed_hsa,
fre = d$freab, #fu HSA
                   clsec =  CLSEC(jmax.t = d$clint,km.t = 1, kwt = d$kw
,mat = d$mat_fr),
                   bp = BP(hemat = d$hemat, fu = d$fu_paed_hsa, kp = d$kp))

d$cl_mat_aag <- CL(qr = d$qr, wt = d$wt, age = d$age, fu = d$fu_paed_aag,
fre = d$freab, # fu AGP
                   clsec =  CLSEC(jmax.t = d$clint,km.t = 1,kwt = d$kw, mat
= d$mat_fr),
                   bp = BP(hemat = d$hemat, fu = d$fu_paed_aag, kp = d$kp))
```

```
# PBPK clearance with mat = 1:
# ---

d$cl_mat_hsa_1 <- CL(qr = d$qr, wt = d$wt, age = d$age, fu = d$fu_paed_hsa,
fre = d$freab, #fu HSA
                    clsec =  CLSEC(jmax.t = d$clint,km.t = 1, kwt = d$kw,
mat = 1),
                    bp = BP(hemat = d$hemat, fu = d$fu_paed_hsa, kp = d$kp))

d$cl_mat_aag_1 <- CL(qr = d$qr, wt = d$wt, age = d$age, fu = d$fu_paed_aag,
fre = d$freab, # fu AGP
                    clsec =  CLSEC(jmax.t = d$clint,km.t = 1,kwt = d$kw, mat
= 1),
                    bp = BP(hemat = d$hemat, fu = d$fu_paed_aag, kp = d$kp))

# prediction error (with and without ontogeny):
# ---

d$pe_hsa <- PE(a = d$cl_mat_hsa_1, b = d$cl_mat_hsa)
d$pe_aag <- PE(a = d$cl_mat_aag_1, b = d$cl_mat_aag)

# Systematic bias:
# ---

tiff(paste0(home, wd, "SC_Figure_3_All_drugs_Systematic_bias_per_function_
and_age_min_med_max.tiff"), width = 20, height = 10, units = "cm", res =
300)

d %>%
  # changes to ds:

  filter(lab != "Adult") %>% filter(transp != "mat" & clint != 0) %>% #mat ==
1 GFR only drugs (clint = 0) are excluded
  melt(id.vars = names(d)[c(-22, -23)], variable.name = "plasma_prot", val-
ue.name = "pe") %>% # all pes in 1 column
  group_by(transp, mat_fr, lab) %>% summarize(maxPE = max(pe), medPE = me-
dian(pe), minPE =min(pe)) %>% # summary stats on %pe for each age and tram-
sp
  mutate(peCol = ifelse(maxPE <=30, 1, ifelse(maxPE > 30 & maxPE <=50, 2,
3))) %>% # colour tiles

  ggplot(aes(x = factor(lab, levels = c("1 Day", "1 Month", "3 Months",
"6 Months", "9 Months", "1 Year", "2 Years", "5 Years", "10 Years", "15
Years")), y = factor(transp))) +
    geom_tile(aes(fill = peCol, alpha = 1, colour=1))+
    scale_fill_gradientn(colours = c("green", "orange", "red"))+
    geom_text(aes(label = paste(round(minPE, 0),"%", "\n",round(medPE,
0),"%","\n", round(maxPE, 0),"%")), size = 3)+
    scale_y_discrete(labels = c("ATS ont. (De Woskin)", "ATS ont. (Hay-
ton)", "P-gp ont.(Cheung)", "OAT1 ont.(Cheung)", "OAT3 ont.(Cheung)", "OCT2
ont.(Cheung)"))+
    theme_bw()+
    xlab("")+
    ylab("")+
    guides(col = FALSE, alpha = FALSE)+
    theme_cowplot(12)+
    theme(panel.spacing=unit(.05, "lines"),
          panel.border = element_rect(color = "black", fill = NA, size =
1.2),
          legend.position = "none")

dev.off()
```

```
# Script: General functions for PBPK Framework - Renal
# Version: 2
# Last Update: 6-2-2020
# Author: SC
#==============================
#
# Renal blood flow (Qr):
#======================
## This is dependent on body surface area, so there is a function for BSA
here too:
# references names used
BSA <- function(ht, age, wt) { # age in years
  haycock <- 0.024265 * ht**0.3964 * wt**0.5378
  dubois <- 0.007184 * ht**0.725 * wt**0.425
  return(ifelse(wt < 15, haycock, dubois)) # in m^2
  # for wt <15kg use Haycock et al. for children < or =15kg, else Dubois
and Dubois
}

## Then add the blood flow (Qr) as a % of CO function:
QR <- function(age, ht, wt) { # CO in L/h if /60
  co <- BSA(ht, age, wt) * (110 + 184 * exp(-0.0378 * age) - exp(-0.24477 *
age)) * 1000 / 60
  return(0.19 * co) # in L/h
}

# this has to be optimize to work differently for males and females:

QR_new <- function(age, ht, wt) { # CO in L/h
  co <- BSA(ht, age, wt) * (110 + (184.974 * (exp(-0.0378 * age) - exp(-
0.24477 * age)))) * 1000 / 60
  fr_qr_male <- 4.53 + (14.63 * age ^ 1.0 / (0.188 ^ 1.0 + age ^ 1.0))
  fr_qr_female <- 4.53 + (13.00 * age ^ 1.15 / (0.188 ^ 1.15 + age ^ 1.15))
  return(rowMeans(as.data.frame(list(fr_qr_male, fr_qr_female)))/100* co) #
in L/h
}

# Kidney weight:
# ---

KW <- function(wt) 1050 * (4.214 * wt ^ 0.823 + 4.456 * wt ^ 0.795) / 1000


# GFR:
#==================
## GFR maturation function (my poster from PAGE 2015 --> Salem 2014); wt is
#in g and age is PMA
## here age should be as PMA (GA + PNA in wks): 40 (wks of GA)+age/7 (if
the age-range is in days)

GFR <- function(wt, age) { # wt in kg and age in years
  return(112 * (((wt * 1000 / 70000) ^ 0.632) * ((40 + age * 365 / 7) ^
3.3) / ((55.4 ^ 3.3) + ( 40 + age * 365 / 7) ^ 3.3)))
} # ml/min

# Calculation of CL determined by GFR (net GFR)
CLGFR <- function(wt, age, fu) { GFR(wt, age) * fu }

# fraction unbound maturation functions:
#---------------------------------------
# HSA (g/l):
HSA <- function(age) {hsa <- 1.1287 * log(age * 365) + 33.746 ; return(h-
```

```
sa)}
fu_paed_hsa <- function(age_ad = 35, age, fu) {return(1 / (1 + (((1 - fu) *
HSA(age)) / (HSA(age_ad) * fu))))}

#alpha1-acid glycoprotein (AAG):
AAG <- function(age) {aag <- 0.887 * (age * 365) ^ 0.38 / ((8.89 ^ 0.38) +
(age * 365) ^ 0.38) ; return(aag)}
fu_paed_aag <- function(age_ad = 35, age, fu) {return(1 / (1 + (((1 - fu) *
AAG(age)) / (AAG(age_ad) * fu))))}

# BP - blood to plasma partition coeficient:
#-----------------------------------------
# hemat is required as input; took from simcyp v.18.r.1
# age in years:
hemat <-function(age) {
  hemat_male <- 53 - ((43.0 * age ^ 1.12 / (0.05 ^ 1.12 + age ^ 1.12)) * (1
+ (-0.93 * age ^ 0.25 / (0.10 ^ 0.25 + age ^ 0.25))))
  hemat_female <- 53 - ((37.4 * age ^ 1.12 / (0.05 ^ 1.12 + age ^ 1.12)) *
(1 + (-0.80 * age ^ 0.25 / (0.10 ^ 0.25 + age ^ 0.25))))
  return(rowMeans(as.data.frame(list(hemat_male/100, hemat_female/100)))) #
in fraction
}

BP <- function(hemat, fu, kp) { bp <<- 1 + hemat * (fu * kp - 1)}

#Secretion clearance and CLint as used in SimCYP mechkim.
# ======================================================

# instead of jmax.t and km.t I will use jmax.t = clint and fix km.t=1
CLSEC <- function(isef.t = 1, jmax.t, km.t, raf = 1,ptcpgk = 60, kwt, mat =
1) # jmax/km is equivalent to ul/min; kwt in g
{ # jmax.t is used interchangeably with clint; dependent on the type of in-
put.
  clsec <- (mat * (isef.t * jmax.t * raf) / km.t) * ptcpgk * kwt / 1000 #
conversion to ml/min from ul/min
  return(clsec) #ml/min
}

# secretion clearance
CL_ACTIV <- function(qr, fu, gfr, clsec, bp){(qr - gfr) * fu * clsec / (qr
+ fu * clsec / bp )} # the function without fu^2; unit ml/min; https://sci-
hub.tw/10.1124/dmd.106.013359

# Well-stirred renal clearance model (double correction) Rowland Yeo (2014)
according to Jamei 2009
CL <- function(qr, wt, age, fu, clsec, bp = 1, fre = 0.01) { # unit is ml/
min
        gfr <- GFR(wt, age)
        clgfr <- CLGFR(wt, age, fu)
        clactiv <- CL_ACTIV(qr, fu, gfr, clsec, bp)
        return(qr * (clgfr / qr + clactiv/qr) * (1 - fre))
}

# Clearances generated using the allometric scaling
CL_AS <- function(cl_ad, wt, wt_ad) {
  cl_ad * (wt / wt_ad) ^ 0.75
}

# Clearances generated using the linear scaling
CL_LIN <- function(cl_ad, wt, wt_ad) {
  cl_ad * (wt / wt_ad)
}
```

```
# Hayton maturation function form 2 days (2.15 kg) till 12 yo (35.5 kg) for
OAT1 #
rfp <- function(wt, age) { # mg/min
  1.08 * (wt ^ 1.04) * exp(-0.185 * (age * 12)) + 1.83 * (wt ^ 1.04) * (1 -
exp(-0.185 * (age * 12)))
}

# -- functions for transporters taken from https://ascpt.onlinelibrary.
wiley.com/doi/epdf/10.1002/cpt.1516
pgp <- function(age) {
  (age * 52) ^ 0.73 / ((age * 52) ^ 0.73 + 4.02 ^ 0.73)
}

oat1 <- function(age) {
  (age * 52) ^ 0.43 / ((age * 52) ^ 0.43 + 19.71 ^ 0.43)
}


oat3 <- function(age) {
  (age * 52) ^ 0.51 / ((age * 52) ^ 0.51 + 30.07 ^ 0.51)
}

oct2 <- function(age) {
  (age * 52) ^ 1 / ((age * 52) ^ 1 + 4.38 ^ 1)
}



# prediction error calculation:
# ----------------------------
PE<-function(a,b) (a-b) / b *100 # a- function to compare; b - function to
compare it to

# calculated allometric scaling coefficient (without GFR maturation func-
tion):
# ------------------------------------------------------------------------
---
coefF <- function(cl_ped, cl_ad, wt_ped, wt_ad) {
  return(log(cl_ped/cl_ad)/log(wt_ped/wt_ad))
}
```