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ARTICLE

Combining data on the bioavailability of midazolam and physiologically-based pharmacokinetic modeling to investigate intestinal CYP3A4 ontogeny

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Abstract

Pediatric physiologically-based modeling in drug development has grown in the past decade and optimizing the underlying systems parameters is important in relation to overall performance. In this study, variation of clinical oral bioavailability of midazolam as a function of age is used to assess the underlying ontogeny models for intestinal CYP3A4. Data on midazolam bioavailability in adults and children and different ontogeny patterns for intestinal CYP3A4 were first collected from the literature. A pediatric PBPK model was then used to assess six different ontogeny models in predicting bioavailability from preterm neonates to adults. The average fold error ranged from 0.7 to 1.38, with the rank order of least to most biased model being No Ontogeny < Upreti = Johnson < Goelen < Chen < Kiss. The absolute average fold error ranged from 1.17 to 1.64 with the rank order of most to least precise being Johnson > Upreti > No Ontogeny > Goelen > Kiss > Chen. The optimal ontogeny model is difficult to discern when considering the possible influence of CYP3A5 and other population variability; however, this study suggests that from term neonates and older a faster onset Johnson model with a lower fraction at birth may be close to this. For inclusion in other PBPK models, independent verification will be needed to confirm these results. Further research is needed in this area both in terms of age-related changes in midazolam and similar drug bioavailability and intestinal CYP3A4 ontogeny.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Studies have been performed to investigate and model the ontogeny profile of intestinal CYP3A but there is uncertainty about the optimal profile to include in p-PBPK models.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study used a p-PBPK model and published pediatric midazolam bioavailability data to assess the different published ontogenies of intestinal CYP3A and determine which was best performing.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

There is a definite ontogeny for intestinal CYP3A enzymes between neonates and older children. Based on the current analysis the best-performing model could be further optimized in terms of fraction at birth and speed of onset.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

Increasing the confidence in system parameters that are used in p-PBPK models such as intestinal CYP3A ontogeny is important in relation to their use in pediatric drug development and for subsequent regulatory acceptance.

INTRODUCTION

There has been a rapid rise in the application of pediatric physiologically-based pharmacokinetic (p-PBPK) models both clinically and in drug development,[1] the latter being reflected in regulatory submissions involving this approach.[2] Although regulatory bodies are supportive of the use of these models, an appropriate level of model qualification related to the context of use is required. This in turn has generated further research into uncertain parameters and this cycle of model building, evaluation, verification, and application is important for generating robust PBPK models with associated user confidence. For p-PBPK models including and verifying the developmental physiology and enzymes/transporter ontogeny data is important for predicting drug pharmacokinetics (PK) in certain age groups.[3] While many age-changing parameters such as liver size and α1-acid glycoprotein are well defined,[4,5] there are some that require more research including the ontogeny of certain intestinal and hepatic enzymes and transporters.

Pediatric PBPK models alongside available clinical data can be used as a research tool to investigate the development of specific parameters. Recent examples using this approach have resulted in the identification of the developmental changes in (i) hepatic OCT1 ontogeny using morphine PK data,[6] (ii) characterization of the effects of physiological factors on oral drug absorption in pediatrics,[7] and (iii) determination of the likely ontogeny of biliary excretion based on azithromycin, ceftazidime, buprenorphine, and digoxin clearance.[8]

The role of intestinal CYP3A4/5 on the pre-systemic metabolism of drugs has been well documented.[9] Although the total mass of CYP3A4/5 in the intestines is about 1% of that in the liver,[10] its strategic expression at the villous tip in the proximal bowel means that it can make a significant contribution to the overall first pass and oral bioavailability of drugs such as midazolam.[11] Concordant with the liver, enterocytic CYP3A5 is also polymorphically expressed in ~20% to 70% of adults, depending on ethnicity.[12]

Several studies have been conducted on the ontogeny of intestinal CYP3A with varying results depending on the marker of CYP3A. Chen et al.[13] and Fakhoury et al.[14] have investigated the changes in duodenal mRNA and both showed a negative correlation with age and overexpression in the neonate declining to adults. The study by Fakhoury et al.[14] showed a similar negative age-developmental pattern for duodenal CYP3A4 and CYP3A5 mRNA and minimal CYP3A7 mRNA with no developmental pattern. All data were normalized for villin, a marker of enterocyte harvest. In contrast, a study performed by Johnson et al.[15] on 115 fetal and pediatric duodenal samples showed a positive ontogeny (increasing activity) with age. These observations were made on protein abundance measured by Western blotting and in vitro metabolism of testosterone to 6β-hydroxytestosterone, results were normalized for villin. Two recent studies both based on proteomics and measuring multiple enzymes and transporters have shown positive ontogeny patterns for duodenal and jejunal CYP3A4 ontogeny when data are normalized for villin.[16,17] The study of Kiss et al.[17] showed No Ontogeny for ileal CYP3A4 compared with a positive ontogeny in the jejunum and showed No Ontogeny for CYP3A5 in both segments of the GI tract. In contrast, Goelen et al.[16] showed a trend toward a positive duodenal ontogeny profile for CYP3A5 although a statistically significant correlation was not seen.

Midazolam is a useful probe substrate for CYP3A activity and given the low frequency of extensive metabolizers for CYP3A5 in the Western population,[18] it can be used to assess CYP3A4 activity. The mean and median fraction

metabolized (fm) are ~85% and 95%, respectively, in the adult healthy Western population. In two recent studies based on intravenous (iv) midazolam and other CYP3A4 drug data in pediatrics from term birth onward to adults,[19,20] the modified hepatic CYP3A4 ontogeny of Upreti and Wahlstrom,[21] was shown to better predict the impact of age on clinical PK data compared with that of Salem et al.[22] As a parallel study, using available oral midazolam bioavailability data in neonates to adults should allow assessment of the published ontogeny patterns for intestinal CYP3A.

Accordingly, given the uncertainty around the ontogeny for intestinal CYP3A enzymes the aims of this study are to:

- Perform a detailed literature search on the oral bioavailability of midazolam in adults and children.
- Test the different derived ontogeny models for intestinal CYP3A to see which best describes the oral midazolam clinical data.
- Investigate the likely expression of intestinal CYP3A in preterm neonates.

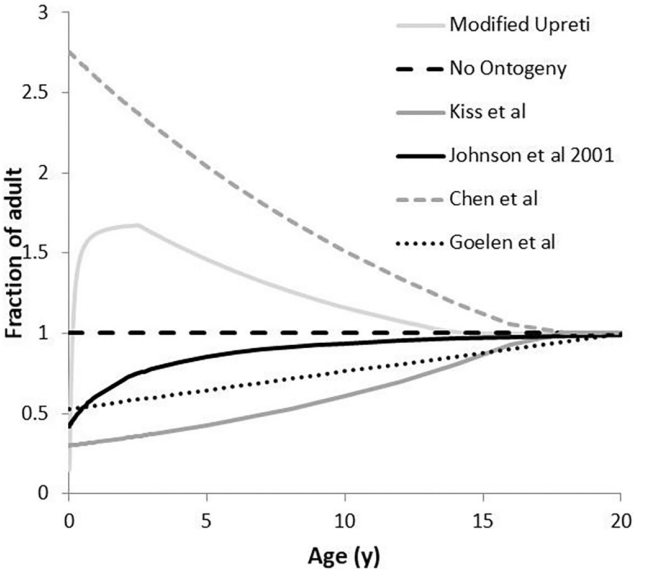

FIGURE 1 Summary of the different published ontogeny profiles for intestinal CYP3A4 (term neonate to adult). Black line is Johnson et al.,[15] Black dashed line is No Ontogeny, Dark gray line is Kiss et al.,[17] Dark gray dashed line is Chen et al.,[13] dotted black line is Goelen et al.[16] and Light Gray line is modified Upreti and Wahlstrom[21] hepatic CYP3A4 ontogeny.

METHODS

Literature search

A detailed literature search was performed using Pubmed and the search terms (Human) AND (Adult OR Pediatric OR Pediatric) AND (midazolam) AND (Pharmacokinetics OR Bioavailability OR Oral). An inhouse library was also searched as were the reference lists of relevant publications. Separate data were compiled for midazolam bioavailability in adult and pediatric subjects. The literature on the ontogeny of intestinal CYP3A had been constantly scanned previously using Pubmed search terms (CYP3A OR CYP3A4 OR CYP3A5) AND (Intestine OR Intestinal OR Gut) AND (Ontogeny OR Development OR Pediatrics OR Pediatrics) and the various models were available in the inhouse library.

Intestinal CYP3A ontogeny models

The published studies and modeling approach are summarized in Table S1 and Figure S1 and a comparison of the derived models is shown in Figure 1, in total six ontogeny models were tested: Johnson et al.,[15] "No Ontogeny" (adult expression from birth), Kiss et al.,[17] Chen et al.,[13] Goelen et al.,[16] and modified Upreti and Wahlstrom[21] (See Supplementary – Modified Upreti Ontogeny for details). The latter was included as a recent study has suggested that this hepatic CYP3A4 ontogeny could also be optimal for intestinal CYP3A ontogeny.[23] The study of Fakhoury et al.[14] was not included as the mRNA results were more extreme but broadly in agreement with those of Chen et al.[13]

Model development

All simulations were performed in Simcyp V22 (Certara UK Limited). The default Sim-Midazolam file incorporating minor elimination via CYP3A5 and UGT1A4 was used in all simulations from term birth onwards. A simplified version of this file was also used to develop the initial study ideas, where all hepatic and intestinal metabolism was assumed to be via CYP3A4. A global CLint value for this enzyme of 3.23 μL/min/ρmol of isoform (based on a mean CLiv of 25.6 L/h, default in Sim-Midazolam file) was incorporated into the compound file using the reverse translation tools within Simcyp. CYP3A7 is highly expressed in preterm neonates[24] and may have an important role in midazolam kinetics in these populations, thus a modified midazolam file incorporating this enzyme was used for preterm simulations, as previously described.[19]

PBPK modeling approach

The first-order absorption model was used to test the different ontogeny models for intestinal CYP3A4/3A5. This

approach uses the Q_{gut} model to determine intestinal metabolism and F_G as shown in Equations 1 and 2, this approach has been previously described in detail.[25]

$$Q_{Gut}=\frac{CL_{perm}\times Q_{ent}}{CL_{perm}+Q_{ent}}, \quad (1)$$

where Q_{Gut} is a hybrid value, where Q_{ent} represents the villous blood flow and CL_{perm} is the clearance permeability of the drug (estimated from in vitro data, for example, Caco-2 cells).

$$F_G=\frac{Q_{Gut}}{Q_{Gut}+fu_{Gut}\times CLu_{int.Gut}} \quad (2)$$

where F_G is the fraction escaping metabolism in the intestine and $CLu_{int.gut}$ is the unbound gut intrinsic clearance value, in this case for CYP3A4/CYP3A5 and fu_{gut} is the fraction unbound within the enterocyte (1 in this case). In the pediatric PBPK model, the CL_{perm} is calculated from the permeability and age-related small intestinal surface area, Q_{ent} is calculated as 6% of the age-changing cardiac output and $CLu_{int,gut}$ is the adult entered value multiplied by the intestinal ontogeny for that enzyme and relative small intestinal surface area for each age. Within the simulations, the F_G was multiplied by the F_H (calculated using in vitro in vivo extrapolation incorporating the relevant hepatic ontogeny profile, and the well-stirred liver model) and F_a (fixed at 1) to calculate the age-related changes in bioavailability (F). The optimal hepatic CYP3A4 ontogeny was fixed as that of the modified Upreti and Wahlstrom[21] (with modification of fraction at birth = 0.15, Age Cap 2 = 12.5 year and removal of C3 parameter – see notes after Table S1) and hepatic CYP3A5 was assumed to have No Ontogeny based on a recent publication.[19]

Simulations trial design

Simulations in pediatrics were performed using the SIM-Pediatric and in preterm using the SIM-Preterm populations. For the simplified midazolam compound model, simulations in pediatrics for each intestinal CYP3A4 ontogeny were performed twice in 1000 subjects, 50% male and female, 0–25 years, firstly with physiological variability set to zero (All CV values set to zero) and then with default variability included. A fixed seed was used in the simulator so that the same individual subjects were repeated between simulations. Simulations were performed twice to construct the figures.

For simulations incorporating CYP3A5 as well as CYP3A4 into the metabolism of the midazolam, the same intestinal ontogeny was applied to both enzymes. To separate the contribution of the two different enzymes, simulations were run without variability in 830 male and female subjects with phenotype for CYP3A5 set to Poor Metabolizer (PM) based on the CYP3A5 PM frequency in healthy Western Caucasian population = 0.83. Simulations were run again without variability in 170 male and female subjects with phenotype for CYP3A5 set to extensive metabolizer (EM). For the 170 subjects common to both simulations, a weighted average, age-related bioavailability (F) was calculated for the Caucasian population CYP3A4/CYP3A5 mix (Equation 3).

$$\text{Individual Weighted average } F_{EM,PM} = F_{EM}\times 0.17 + F_{PM}\times 0.83 \quad (3)$$

Finally, a simulation was run in 1000 male and female subjects with CYP3A5 phenotype set to 0.83 for PM and 0.17 for EM with variability included. These multiple simulations were performed to generate the figures, separating the influence of CYP3A4, CYP3A5 (PM & EM), and variability.

For comparison, the best-performing intestinal CYP3A4 ontogeny model using the first-order absorption model was also run with the pediatric advanced dissolution, absorption and metabolism (p-ADAM) model.[26] In this case, a crossover design was used with all 1000 subjects (prop females = 0.5, 0 to 25 year, PM for CYP3A5 = 0.17, dose = 0.5 mg/kg given intravenously) simulated in first-order and p-ADAM oral models, in all cases CV values were set to default and variability included. The ratio of $AUC_{0\text{-inf}}$ values (oral/iv) was used to calculate the impact of age on bioavailability.

The preterm simulations were matched as closely as possible to the two clinical studies in terms of the number of subjects, gestational, and postnatal age. For the de Wildt study[27] the multiple population trial design was used with differing gestational ages (GA) set to 26, 28, and 31 weeks and postnatal age (PNA) set to 8 days. For the study by Brusse et al.,[28] the multiple population trial design was also used with GA of 26, 30, and 34 weeks and PNA set to 7 days. For both simulations, an equal number of subjects per sub-population was assumed. The default hepatic CYP3A4 (same assumed for CYP3A5) and CYP3A7 ontogeny were applied in all cases.[29] The ontogeny for intestinal CYP3A4/5 was varied from zero (no expression) up to 1 (adult expression) to replicate the reported bioavailability in each clinical study.

Data analysis

The adult midazolam bioavailability data were combined to give a global weighted mean and standard deviation as previously described.[30] Less weighting for number of subjects was applied to the three studies conducted

using population PK (POPPK) rather than a standard iv to oral crossover design (see footnote Table S2). The predicted changes in F (with and without added physiological variability with age were visually compared against the available clinical studies for each of the intestinal CYP3A ontogeny profiles). Prediction accuracy for age-specific F values was based on Equations 4 and 5 used to calculate the average fold error (AFE) and absolute average fold error (AAFE) as measures of bias and precision respectively.[31]

$$\mathrm{AFE} = 10^{\frac{1}{n}\sum \log \frac{\mathrm{Pred}}{\mathrm{Obs}}} \quad (4)$$

$$\mathrm{AAFE} = 10^{\frac{1}{n}\sum \left|\log \frac{\mathrm{Pred}}{\mathrm{Obs}}\right|} \quad (5)$$

RESULTS

Literature search

In total, 36 studies were found giving values for the bioavailability of midazolam in adults, some studies provide multiple values based on sex, dose, ethnicity, CYP3A5 genotype, formulation, and renal dialysis and these are shown in Table S2 and Figure 2. The range of values was 0.096–0.68; however of the 54 values, only six were outside the range of 0.2–0.45 with a weighted mean value of 0.29. Only two studies considered the CYP3A5 genotype and six had information on ethnicity.

Only six studies were found reporting midazolam F values across the pediatric age range, two of these were in premature neonates as shown in Table S3. A total of 13 values were reported in discrete age bands and these are shown with postmenstrual age (PMA) in comparison to the weighted adult values in Figure 3 and Figure S2 (log scale). After the neonatal/early infant period most reported values were in a similar range compared with adult values.

Comparison of the possible intestinal CYP3A4/CYP3A5 ontogeny

A comparison of the six tested ontogeny models based on the simplified midazolam model, with metabolism via CYP3A4 only, is shown in Figure 4 and Figure S3 (log scale). Visually comparing the mean simulated values across the PMA with the observed values, the models of Johnson and modified Upreti and Wahlstrom (Upreti) appear to perform best and capture the early PMA studies. After the neonatal and infant ages (around 100 weeks PMA) the difference between all models, except for Kiss, appears marginal. The model of Chen based on mRNA data underpredicts F in the very young while that of Kiss overpredicts in some of the infant ages. The predictions of F for the individual study age ranges for the different ontogeny models as shown in Table S4 and the AFE and AAFE values are shown in Table 1. The AFE ranges from 0.7 to 1.38 and the rank order of least to most bias model is No Ontogeny < Upreti = Johnson < Goelen < Chen < Kiss. The AAFE ranges from 1.17 to 1.64 and the rank order most to least precise is Johnson > Upreti > No Ontogeny > Goelen > Kiss > Chen.

Comparing the same models but incorporating CYP3A5 into the midazolam compound file (Figure 5 and Figure S4 on log scale) increases the level of uncertainty but visually

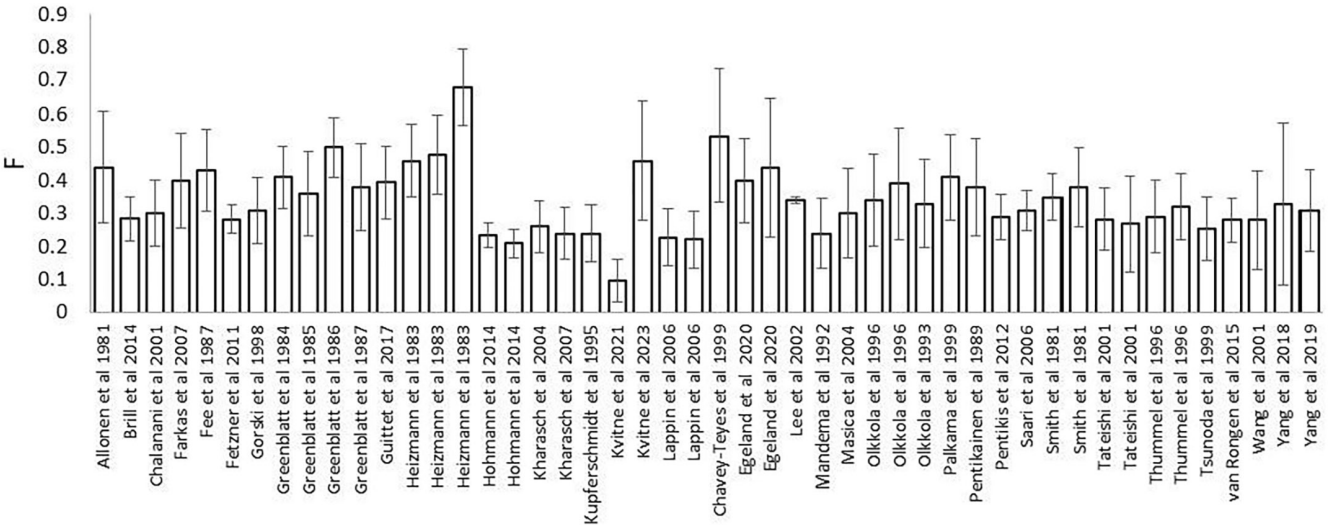

FIGURE 2 Summary of the published data on the bioavailability of midazolam in adults used in the Meta-analysis. For each study, the mean values and error bars (±SD) are shown. For some studies, male and female values were combined. For full list of references see Table S2.

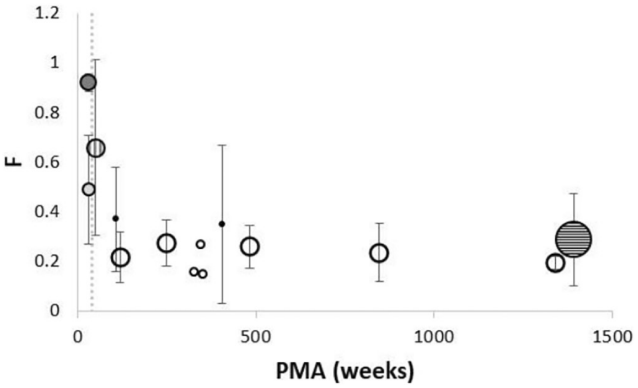

FIGURE 3 Summary of the published data on the bioavailability of midazolam in neonates, infants, and children in relation to post-menstrual age (PMA). The circles with different fills represent different studies, black horizontal stripe is data from adult meta-analysis, no fill is Brussee et al.[38] black fill is Reed et al.,[35] white fill is Payne et al.,[34] light gray fill is de Wildt et al.,[27] dark gray vertical stripe is von Groen et al.[36] and dark gray is Brussee et al.[28] and their size reflects the size of the study population. The vertical gray dashed line at 40 weeks PMA distinguishes studies mainly performed in preterm and from term neonates.

the No Ontogeny, Johnson, Goelen, and Upreti models appear to perform best with most of the observed data points between the CYP3A5 PM and EM moving average lines. Again, the model of Chen based on mRNA data underpredicts F in the very young while that of Kiss overpredicts in some of the infant ages. The predictions of F for the individual study age ranges for the different ontogeny models are shown in Table S4 and the AFE and AAFE values are shown in Table 1. The AFE ranges from 0.69 to 1.48 and the rank order of least to most bias model is No Ontogeny < Upreti < Johnson = Goelen < Chen < Kiss. The AAFE ranges from 1.39 to 1.56 and the rank order most to least precise is Johnson = Upreti > No Ontogeny > Goelen > Kiss = Chen. The rank order was similar between each of the two midazolam compound files but particularly when including CYP3A5 the difference in the performance of the best-performing four models was marginal. Taking the Johnson model as one of the best-performing models, a comparison was made in bioavailability prediction between the first-order and p-ADAM models in Simcyp using the default Sim-Midazolam file. The results are shown together with PMA in Figure 6a alongside observed data, it should be pointed out a different method was used to calculate F in this case compared with previously (Figure 5) and there is some slight overprediction. However, as expected there remains a high correlation between the two absorption models $r^2 = 0.94$ (Figure 6b).

The observed bioavailability for the two preterm studies performed in a similar GA and PNA range were 0.49[27] and 0.92[28] with a weighted mean of 0.80. Using the preterm population model in Simcyp with the hepatic fractional ontogeny for CYP3A4 and CYP3A5 and CYP3A7 set to default, the simulated bioavailability was 0.94, 0.77, and 0.62 assuming an intestinal CYP3A4/5 ontogeny fraction of 0, 0.42, and 1, respectively. To capture the bioavailability of 0.49 the intestinal CYP3A4/5 ontogeny was set to 1 (adult expression) and the hepatic CYP3A4 ontogeny fraction had to be increased from 0.077 to 0.35, the latter value is higher than the default value for term neonates of 0.15.

DISCUSSION

There was significant variability in the bioavailability reported in adult studies with values ranging from 0.096 to 0.68 with a weighted mean of 0.29. Only two studies considered the effects of CYP3A5 genotype, Karasch et al.[32] showed a modest effect with $F = 0.26$ and 0.22 in PM and wild-type EM, respectively while Kvitne et al.[33] reported a relatively high value of 0.46 in CYP3A5 PMs after excluding subjects with *1 alleles. In the present study, the simulated values in pure PM or EM adult subjects were 0.34 and 0.17, respectively based on the Sim-Midazolam compound file. This study predicts a more significant effect of CYP3A5 phenotype than that observed in the clinical studies. Further studies are needed to investigate the influence of CYP3A5 on midazolam bioavailability.

Only six studies were found reporting the bioavailability of midazolam in children, four were based on a crossover design, one on a parallel group design and the other on a population PK approach. The difficulty of performing crossover bioavailability studies in neonates and young children is acknowledged. None of the studies considered the effects of CYP3A5 and this could have been significant in the studies by Payne et al.[34] and Reed et al.[35] as these were performed in South African and US populations respectively. Unfortunately, neither study reported subject ethnicity and it is well known that Black African/African American populations have a much higher proportion of CYP3A5 EM phenotypes.[18] In general, after 1 year of age the mean bioavailability was at adult values although some of the studies observed significant variability.[35] Reported bioavailability was highest in the studies performed in premature neonates,[27,28] and term neonates and infants,[36] the latter study was performed in stable but critically ill subjects and some may have had CYP3A4 suppression due to inflammation.[37] Other limitations of the current pediatric midazolam bioavailability data include the lack of values in the 0–2-year-old age range separated into discrete age categories. Additionally, unlike the adult data which is predominantly obtained from healthy subjects, pediatric studies are performed in children with disease and the impact of this cannot be ignored.[37]

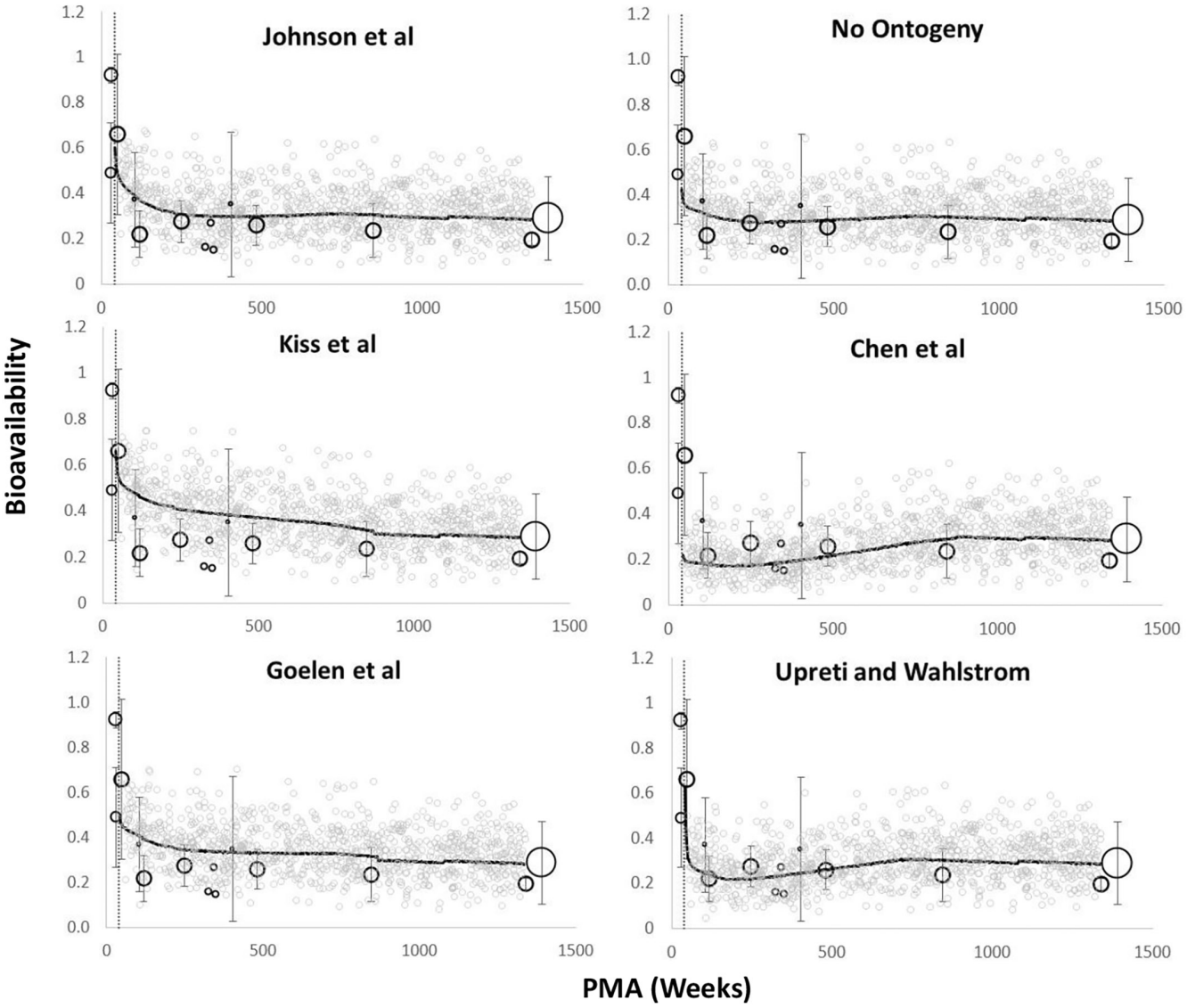

FIGURE 4 Predicted age-related bioavailability using the different intestinal CYP3A ontogeny profiles for a midazolam compound file assuming all CYP3A4 metabolism. Gray open circles are simulated values from 1000 subjects aged 0–25 years (40–1340 Weeks PMA), solid black line is the mean simulated value (all variability set to zero, akin to a moving average), the open black circles are the clinical studies, and their size reflects the number of subjects, error bars are ±SD. The vertical black dotted line is at 40 weeks PMA and divides studies performed in preterm and term neonates.

Midazolam is a BCS class 1 compound where absorption is likely to be rapid and complete. Given that the ontogeny of the hepatic metabolism via CYP3A4 and CYP3A5 is relatively well understood,[19] oral midazolam bioavailability was considered as a possible marker to assess the intestinal ontogeny of CYP3A. Two separate midazolam models with and without CYP3A5 were presented in this paper to try to add clarity and delineate the effects of this enzyme.

Two of the studies[13,14] investigating the ontogeny of intestinal CYP3A showed an overexpression of mRNA at birth which then declines to adult levels. While this may be the case, this study suggests this overexpression does not translate into higher enzyme activity at birth as the Chen model showed high bias and low precision. Four of the ontogeny models are based on protein abundance[15–17] but have different fractions at birth values and different trajectories, the Kiss ontogeny is based on their data for the jejunal whereas the "no ontogeny model" is based on their data from ileum.[17]

Several limitations and assumptions exist regarding the compared ontogeny models, not least the relatively small number of wide age groups reported in some studies.[17] The study by Goelen et al.[16] only included data down to 11 months of age and so below this age, the ontogeny is extrapolated linearly back to birth. The modified Upreti

TABLE 1 Summary of the performance of the different ontogeny models for intestinal CYP3A against available pediatric values for *F*.

	CYP3A4 Midazolam model		CYP3A4 and CYP3A5 Midazolam model	
Ontogeny model	**AFE**	**AAFE**	**AFE**	**AAFE**
Johnson et al.	1.04	1.17	1.28	1.39
No Ontogeny	1.02	1.35	1.10	1.40
Kiss et al.	1.38	1.49	1.48	1.56
Chen et al.	0.7	1.64	0.69	1.56
Goelen et al.	1.22	1.39	1.28	1.41
Modified Upreti and Wahlstrom	0.96	1.33	1.12	1.39

Note: *CYP3A4 midazolam model.*

AFE (Bias) including all studies No Ontogeny < Upreti = Johnson < Goelen < Chen < Kiss.

AAFE (Precision) including all studies Johnson > Upreti > No Ontogeny > Goelen > Kiss > Chen.

CYP3A4 and CYP3A5 Sim-Midazolam model.

AFE (Bias) including all studies No Ontogeny < Upreti < Johnson = Goelen < Chen < Kiss.

AAFE (Precision) including all studies Johnson = Upreti > No Ontogeny > Goelen > Kiss = Chen.

ontogeny is extrapolated from a hepatic CYP3A4 ontogeny.[21] A recent PBPK study[23] on predicting tacrolimus disposition in pediatric subject concluded that this was the best-performing CYP3A4 ontogeny in both liver and gut and hence the reason for evaluating it in this study. Conflicting evidence exists for this intestinal ontogeny pattern, in vitro studies on intestinal CYP3A4 mRNA suggest overexpression in pediatrics compared with adults but protein expression and activity studies do not support this. A physiological POPPK study by Brussee et al.[38] including midazolam data from 264 postoperative children found a higher intestinal intrinsic clearance per gram intestine in 1-year olds compared with older children. The data to support this finding are largely dependent on the physiological parameter estimations included in the model; however, the calculated values for *F* with age agree with other studies. A criticism of this study is that given the strategic expression of the enzyme in the gut wall, data would have been better presented as intrinsic clearance per gut surface area rather than per gram intestinal weight. Previous studies have shown separate regulation of intestinal and hepatic CYP3A enzymes in humans which may translate into different ontogeny patterns.[39]

For the Sim-Midazolam compound file incorporating CYP3A5, it was assumed that intestinal CYP3A4 and 5 both had the same ontogeny, based on visual inspection of the presented data, two of the available in vitro studies visually show a similar ontogeny for both enzymes[16,17] but did not report a statistically significant correlation for CYP3A5 with age. Overall, the expression of CYP3A7 appears to be very low in the intestine, in infants the mRNA/villin ratio was reported as 2500-fold lower than for CYP3A4, its contribution to enterocytic metabolism was described as limited[14] and is thus ignored in this study. However, the contribution of hepatic CYP3A7 to overall midazolam bioavailability was considered in preterm neonates.

Visually the Johnson, Upreti, and No Ontogeny models appear to perform best. Overall, the Johnson model was the most precise, and the No Ontogeny model showed the least bias regardless of the midazolam model. However, the results were marginal and it was difficult to discern the most optimal model particularly when the effects of CYP3A5 were factored into the midazolam compound model. In terms of precision, the Johnson, Goelen, Upreti, and No ontogeny models all gave similar values for AAFE. The AFE and AAFE values are calculated for mean data, when physiological variability is added to the PBPK model, including for both hepatic and intestinal determinants of *F*, distinguishing the best-performing models becomes even harder. Making the Johnson ontogeny faster with a lower value at the time of birth[17,21] would potentially improve its performance, this pattern is suggested by a recent study.[40]

The bioavailability of midazolam in a similar cohort of preterm neonates (GA and range of PNA) is reported to be 0.49[27] and later as 0.92.[28] In both studies, subjects were in neonatal intensive care but not on drugs likely to affect CYP3A4 or reported having significant underlying disease. The discrepancy is likely due to the different methodologies used between the observed preterm PK studies, de Wildt et al.[27] used a crossover design, whereas Brussee et al.[28] used a physiological population PK model incorporating the ontogeny of some physiological parameters such as tissue volumes and protein binding. In our investigation, only using the default preterm hepatic CYP3A4 (& CYP3A5) and CYP3A7 ontogeny and assuming no intestinal CYP3A4/5 expression was a value for *F* of 0.94 simulated. In contrast, to recover a value of 0.49 intestinal CYP3A4/5 ontogeny had to be set to 1 as well

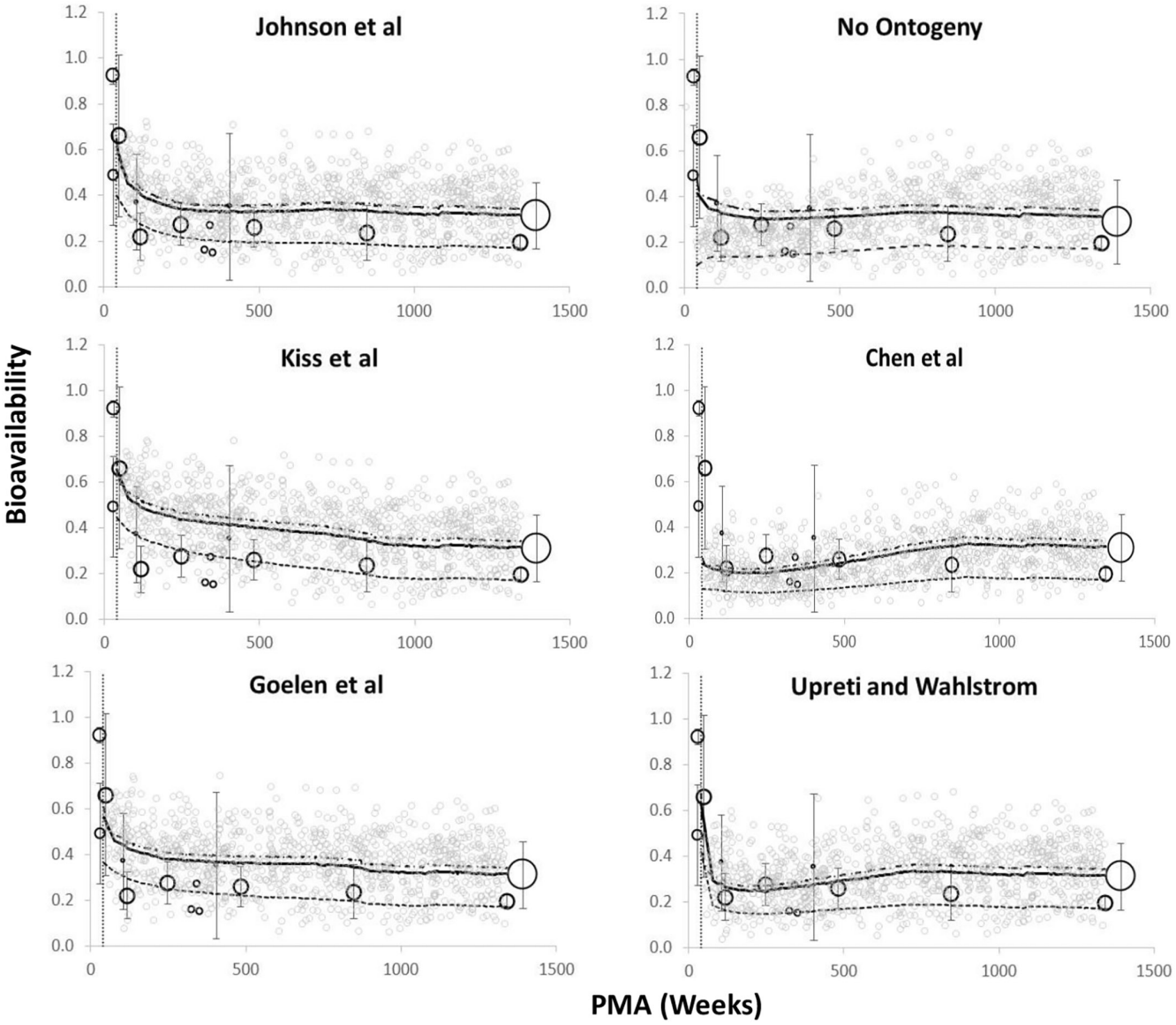

FIGURE 5 Predicted age-related bioavailability using the different intestinal CYP3A ontogeny profiles for the default Sim-Midazolam file incorporating CYP3A4 and CYP3A5 metabolism. Gray open circles are simulated values from 1000 subjects aged 0–25 years (40–1340 Weeks PMA), solid black line is the mean simulated value for a mixed CYP3A4/3A5 Western population (all variability set to zero, akin to a moving average), dashed–dotted black line is for CYP3A5 PM population and dashed black line is for a CYP3A5 EM population, the open black circles are the clinical studies, and their size reflects the number of subjects, error bars are ±SD. The vertical black dotted line is at 40 weeks PMA and divides studies performed in preterm and from term neonates.

as increasing hepatic expression of the enzymes above that currently used for term neonates. What this study does show is increased bioavailability of oral midazolam in preterm neonates but in these populations, the intestinal CYP3A ontogeny remains uncertain, a recent study has shown lower but variable expression in preterm neonates.[40] Further research is needed on oral absorption and both intestinal and hepatic CYP3A expression in preterm neonates and on how these changes impact the bioavailability of midazolam.[41]

The current study utilizes the Q_{gut} model which assumes a unified expression of intestinal CYP3A4/5 through the length of the gut, in the pediatric model the overall adult intestinal expression is corrected using the ontogeny, and both Q_{gut} and CLint are corrected by relative gut surface area compared with adults. This Q_{gut} model was compared with the ADAM model[42] which divides the gastrointestinal tract into nine segments each with their own blood flow and CYP3A4/5 expression with physiological distribution of these enzymes more toward the duodenum and jejunum as found physiologically. The intestinal metabolism is calculated in each gut segment rather than using the Q_{gut} model. Within the p-ADAM model[26] the ontogeny of various GI parameters

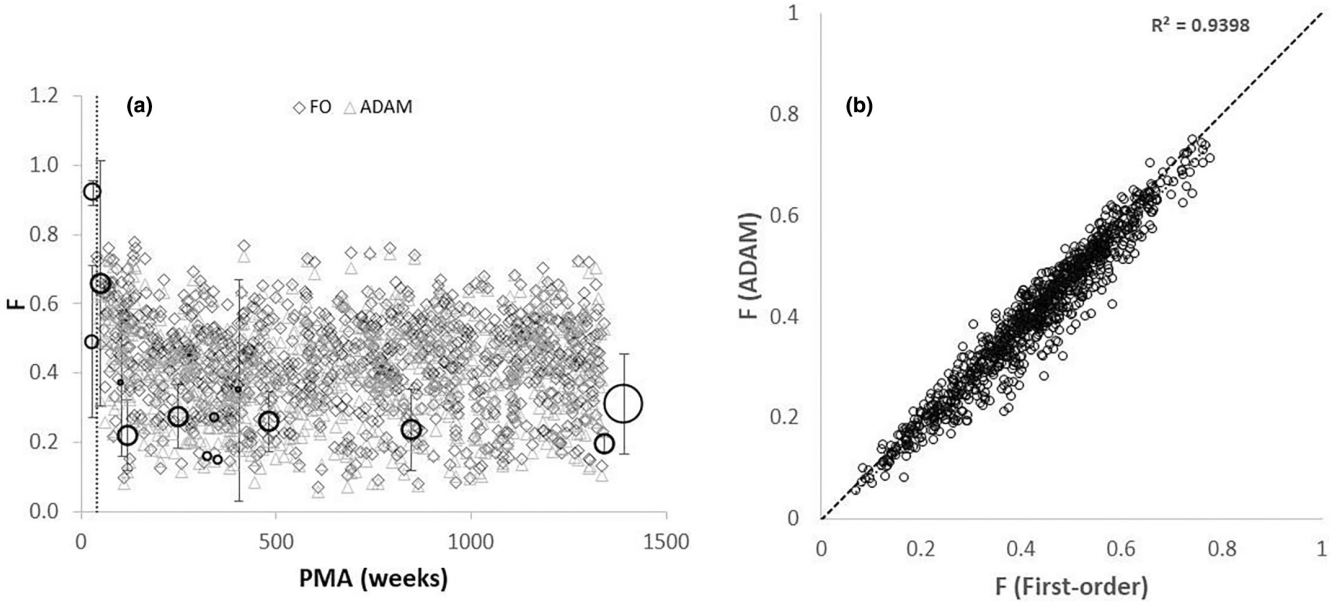

FIGURE 6 Comparison of the prediction of the bioavailability of oral midazolam in pediatrics using the first-order and ADAM models. (a) Direct comparison of the two models against PMA, black diamonds are for first-order model and gray triangles are ADAM model, dark black circles are the clinical data. (b) Correlation for predicted bioavailability between the two models.

is defined; gut metabolism is again corrected for relative gut surface area and intestinal enzyme ontogeny. A similar bioavailability was predicted across the pediatric age range using both absorption models. One advantage of the ADAM model is that it could be expanded to allow the inclusion of the ontogeny of enzymes in different segments of the GI tract, as measured in the study by Kiss et al.[17]

Other areas for further research relate to the strategic expression of the intestinal CYP enzymes and the interplay between enterocyte turnover and enzyme expression with age. The CYP expression within the enterocytes is key and there is some evidence that in subjects less than 6 months old, only certain enterocytes seem to express the enzyme.[14]

Although there is increasing evidence for the improved performance[19,20,23,43] of the modified Upreti[21] hepatic CYP3A4 ontogeny compared with that of Salem et al.,[22] some studies have failed to reach a firm conclusion.[44–46] Any error here will impact the evaluation of intestinal CYP3A ontogeny and remains a limitation for the current study.

In conclusion, there is a definite ontogeny for intestinal CYP3A in neonates and infants but there appears to be little age-related change beyond 1 year of age. The optimal ontogeny model is difficult to discern after considering the effects of CYP3A5 and other population variability. Given the available data, the modified hepatic CYP3A4 ontogeny of Upreti et al.[21] and the intestinal CYP3A ontogeny of Johnson et al.[47] will be retained in the Simcyp model for term neonates and older. Future development of this intestinal CYP3A4 ontogeny model could include optimization in terms of speed of onset and fraction at birth. For inclusion in other PBPK software models the user should perform their verification, particularly if the hepatic ontogeny for CYP3A4 is different. Improving the current study requires additional information both on midazolam and other specific CYP3A4 drug bioavailability in pediatrics and on intestinal CYP3A ontogeny, particularly in the early age groups.

AUTHOR CONTRIBUTIONS

T.N.J., H.K.B., J.G., R.D.H. and J.D. wrote the manuscript. T.N.J. and H.K.B. designed the research. T.N.J. and J.G. performed the research. T.N.J. and J.D. analyzed the data.

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TNJ and JD are employees of Certara UK Limited and may hold shares in Certara. All other authors declared no competing interests for this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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