

## ARTICLE

# Could Postnatal Age–Related Uridine Diphosphate Glucuronic Acid Be a Rate-Limiting Factor in the Metabolism of Morphine During the First Week of Life?

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Neonates experience dramatic changes in the disposition of drugs after birth as a result of enzyme maturation and environmental adjustment, challenging therapeutic decision making. In this research, we establish postnatal age, postmenstrual age, and body weight as physiologically reasonable predictors of morphine's clearance in neonates. By integrating knowledge of bilirubin, morphine, and other drugs metabolized by glucuronidation pathways from previously published studies, we hypothesize that uridine diphosphate glucuronic acid, a postnatal age–dependent sugar, plays an important role in the metabolism of morphine during the first week of life. This finding can be extended to other drugs metabolized by uridine diphosphate glucuronosyltransferase pathways in neonates and thus has important clinical implications for the use of drugs in this population.

## Study Highlights

### WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Morphine is mainly eliminated by glucuronidation through the uridine diphosphate glucuronosyltransferases 2B7 pathway. Morphine metabolism in neonates and infants is predominantly driven by changes in body weight and enzyme maturation as a function of postmenstrual age.

### WHAT QUESTION DID THIS STUDY ADDRESS?

☑ A postnatal age–dependent morphine metabolism is also identified in neonates in addition to body weight and postmenstrual age. This study addresses the potential physiological reason for the postnatal age–dependent morphine metabolism in neonates and its clinical implication.

### WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Postnatal age–dependent glucuronidation activity was found in neonates. Based on literature evidence, we hypothesize that the lack of uridine diphosphate glucuronic acid may explain the reduced morphine metabolism during the first week of life.

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

☑ Uridine diphosphate glucuronosyltransferase substrates may require dosing adjustment in the first week of life.

Morphine is primarily metabolized by uridine diphosphate glucuronosyltransferases (UGT) 2B7 and is widely used for neonatal pain control and sedation.<sup>1</sup> Morphine is also one of the first-line opioids for the treatment of neonatal opioid withdrawal syndrome, known as neonatal abstinence syndrome (NAS). Neonates who were exposed to opioids *in utero* are at risk for the development of NAS after delivery. In a national survey of the management of *in utero*–acquired NAS, morphine sulfate was by far the most commonly used first-line agent for both opioid and polysubstance withdrawal.<sup>2</sup>

UGTs play a major role in drug disposition through the glucuronidation pathways in humans and account for the metabolism of 10% of the 200 most prescribed medicines in the United States.<sup>3</sup> UGTs are also responsible for the elimination of endogenous substances, such as bilirubin and bile acids. Bilirubin (indirect bilirubin) is exclusively metabolized by UGT1A1 in human into bilirubin mono-glucuronide and di-glucuronide (direct bilirubin).<sup>4</sup> Decreased UGT1A1 activity is responsible for several diseases, including neonatal jaundice and hyperbilirubinemia.<sup>5</sup> The age-dependent expression of different UGTs has been characterized in human liver microsomes.<sup>6–8</sup>

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Received: February 18, 2019; accepted: March 13, 2019. doi:10.1002/psp4.12407

To date, extensive effort has been put into the understanding of morphine disposition in pediatrics, especially neonates. Physiologically meaningful predictors, such as body weight and postmenstrual age (PMA), have been established to describe the pharmacokinetic (PK) characteristics of morphine in pediatrics.<sup>9</sup> Other relevant predictors, such as bilirubin concentration, were also reported.<sup>10</sup> In an earlier publication, we developed a population PK model of morphine after oral administration of diluted tincture of opium (DTO; hereby referred to as the DTO model) in which morphine is the active ingredient.<sup>11</sup>

To further understand the PK of enteral morphine in neonates, two clinical trials in late preterm and full-term neonates treated with enteral morphine for intensive care unit (ICU)-acquired and *in utero*-acquired NAS were included in addition to the data from the DTO model. The objective of this research was to improve the mechanistic understanding of morphine PK in neonates by expanding on the published DTO model.

## METHODS

### Clinical trials

**Johns Hopkins University (JHU)-Morphine trial.** Between the years 2012 and 2014, all women who delivered babies at risk for NAS as a result of *in utero* methadone or heroin exposure were approached for consent at a large, multisite, academic hospital system. This clinical trial was approved by the Johns Hopkins Medical Institution institutional review board. Once parental consent was obtained, the neonates were monitored closely for NAS and were only enrolled in the study if they required opiate therapy. Once started on enteral morphine therapy, the daily times and doses of administration were entered into an electronic database. In addition to *in utero*-acquired opiate withdrawal, the neonates with ICU-acquired NAS as a result of the weaning of medical opioids who were treated with enteral morphine were also eligible for study entry. The dosing strategy was similar to the previously published clinical trial, which is the basis for the DTO model.<sup>12</sup> Briefly, all of the patients started oral morphine sulfate solution at 80 µg every 4 hours. The dosing adjustment was made based on the evaluation of withdrawal symptoms by the modified Finnegan score.<sup>13</sup> If necessary, the dose was up-titrated every 8 hours by 40-µg increments to 200 µg every 4 hours and then to 200 µg every 3 hours to 360 µg every 3 hours. Upon stabilization, the morphine dose was then de-escalated by increments of 20 µg per dose every 24 hours. The neonates underwent heel stick puncture for capillary samples up to four times during the entire study period for collection of drug and metabolite concentrations based on clinical practice convenience, and the samples were refrigerated for less than 24 hours. They were then centrifuged to isolate the plasma, and the plasma samples were frozen at -80°C until batch analysis via High Performance Liquid Chromatography with tandem Mass Spectrometry, HPLC-MS/MS techniques as described in a separate publication.<sup>14</sup>

**Thomas Jefferson University (TJU)-Morphine trial/the Blinded Buprenorphine OR Neonatal Morphine Solution (BBORN) trial (ClinicalTrials.gov identifier NCT01452789).** The trial design was previously described,<sup>15</sup> and the PK-related design aspects are described here. Between the years 2011 and 2016, neonates exposed to opioids *in*

*utero* with more than 37 weeks of gestational age (full term only) were eligible to enroll in the clinical trial if they demonstrated adequate signs and symptoms of NAS and required treatment. Morphine sulfate solution was one of the therapeutic options in this clinical trial. For neonates on morphine sulfate solution, their initial dose was 0.07 mg/kg every 4 hours, and the maximum daily dose was 1.25 mg/kg/day. Morphine dose was up-titrated by 20% and weaned by 10% based on the withdrawal symptoms. The cessation dose was 0.025 mg/kg every 4 hours. Sparse sampling for the evaluation of PK was predefined, including peak and trough concentrations during the first week and second week as well as after dose cessation. Considering the sparse sampling regimen, some allowance for variation from this schedule was anticipated to reflect feeding and sleeping schedules for the neonates. Capillary blood samples were drawn by heel stick with a volume goal of 0.4 mL blood into a lithium heparin tube. The method used for the analysis of morphine was modified from the previously published method.<sup>16</sup> Because a pediatric sample has a limited volume, a 100-µL aliquot was used. The calibration curve was adjusted to 1–250 µg/L, with quality control sample concentrations at 3, 20, and 200 µg/L. The internal standard concentration was 20 µg/L. A 10-mM ammonium carbonate buffer with pH 9.3 was used for solid phase extraction. A shorter high-performance liquid chromatography (HPLC) column (YMC ODS AQ 5 µm 2 × 100 mm) was adopted. The method was cross-validated to a Thermo Scientific—TSQ Quantum instrument (Waltham, MA) with 0.1% formic acid in Milli-Q (Burlington, MA) water and methanol as mobile phase, running isocratic with 92% water and 8% methanol for 6.5 minutes. Reconstitution volume was 75 µL in 100% water. The accuracy and precision of this modified morphine assay were evaluated to be 96.7–106.5% and 2.3–7.7%.

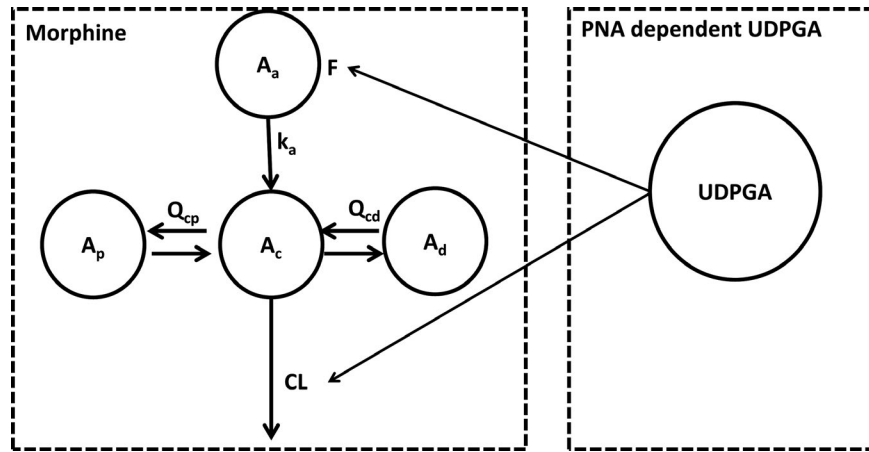
**JHU-DTO trial (ClinicalTrials.gov identifier NCT00510016).** The trial design including the PK aspects and modeling (referred to as the DTO model) were previously reported.<sup>11</sup> Data from the 34 neonates used for the DTO model were also included in the current model development.

### Original DTO model

The DTO model refers to the previously published morphine population PK model after oral administration of DTO.<sup>11</sup> Briefly, a three-compartment PK disposition model of morphine based on rich sampling after intravenous administration in healthy adults was first adopted as the structural model for morphine.<sup>17</sup> Second, body weight-based allometric scaling was added to the central and peripheral volumes of distribution using an exponent of one, and on systematic clearance (CL) and intercompartmental using an exponent of 0.75. Third, the developmental ontogeny for CL was fixed at the previous publication,<sup>9</sup> and the volume of distribution at the physiological change in extracellular water. Detailed information for the original DTO model could be found in Liu *et al.*<sup>11</sup> Fourth, the bioavailability and absorption rate constant were estimated.

### Modified morphine model

In the current research, the DTO model was initially verified using the morphine concentration data collected in the



**Figure 1** Final structural morphine pharmacokinetic model after oral administration of morphine sulfate solution in neonates with abstinence syndrome.  $A_a$ , amount of morphine in the absorption compartment;  $A_c$ , amount in the central compartment;  $A_p$  and  $A_d$ , amounts in the peripheral compartment and deep peripheral compartment; CL, the clearance;  $F$ , bioavailability;  $k_a$ , first-order absorption rate constant; PNA, postnatal age;  $Q_{cd}$ , intercompartment CL between the central compartment and deep peripheral compartment;  $Q_{cp}$ , intercompartmental CL between the central compartment and peripheral compartment; UDPGA, uridine diphosphate glucuronic acid level.

JHU-Morphine study and TJU-Morphine study. In this evaluation, the first-order conditional estimate with interaction method was chosen to evaluate the data with the number of iterations set to 0 to derive individual PK parameters.

Based on the verification, a postnatal age (PNA)-dependent modeling bias was identified (see model verification in the Results section), and the DTO model was modified to fit the raw data from all three clinical trials (JHU-DTO, JHU-Morphine, TJU-Morphine).

The PNA effect was identified to influence either morphine CL or bioavailability ( $F$ ). However, adding PNA effect on only one of the two PK parameters resulted in either a CL of 0 L/hour right after birth or an  $F$  of 100%. Neither of these two situations were physiologically possible for morphine. Therefore, the PNA effect was considered as a change in intrinsic CL. A sigmoid  $E_{max}$ -type model was added on both CL and  $F$  in the DTO structural model to quantify the PNA-dependent presystemic and systemic disposition of morphine after oral administration.

The final structural PK model (Figure 1) is as follows:

$$\frac{dA_a}{dt} = -k_a \cdot A_a \quad (1)$$

$$\frac{dA_c}{dt} = k_a \cdot A_a - \frac{CL \cdot PNA \text{ Effect}}{V_c} \cdot A_c - \frac{Q_{cp}}{V_c} \cdot A_c + \frac{Q_{cp}}{V_p} \cdot A_p - \frac{Q_{cd}}{V_c} \cdot A_c + \frac{Q_{cd}}{V_d} \cdot A_d \quad (2)$$

$$\frac{dA_p}{dt} = \frac{Q_{cp}}{V_c} \cdot A_c - \frac{Q_{cp}}{V_p} \cdot A_p \quad (3)$$

$$\frac{dA_d}{dt} = \frac{Q_{cd}}{V_c} \cdot A_c - \frac{Q_{cd}}{V_d} \cdot A_d \quad (4)$$

$$PNA \text{ Effect} = 1 - \Delta \cdot \left( 1 - \frac{PNA^\gamma}{PNA_{50}^\gamma + PNA^\gamma} \right) \quad (5)$$

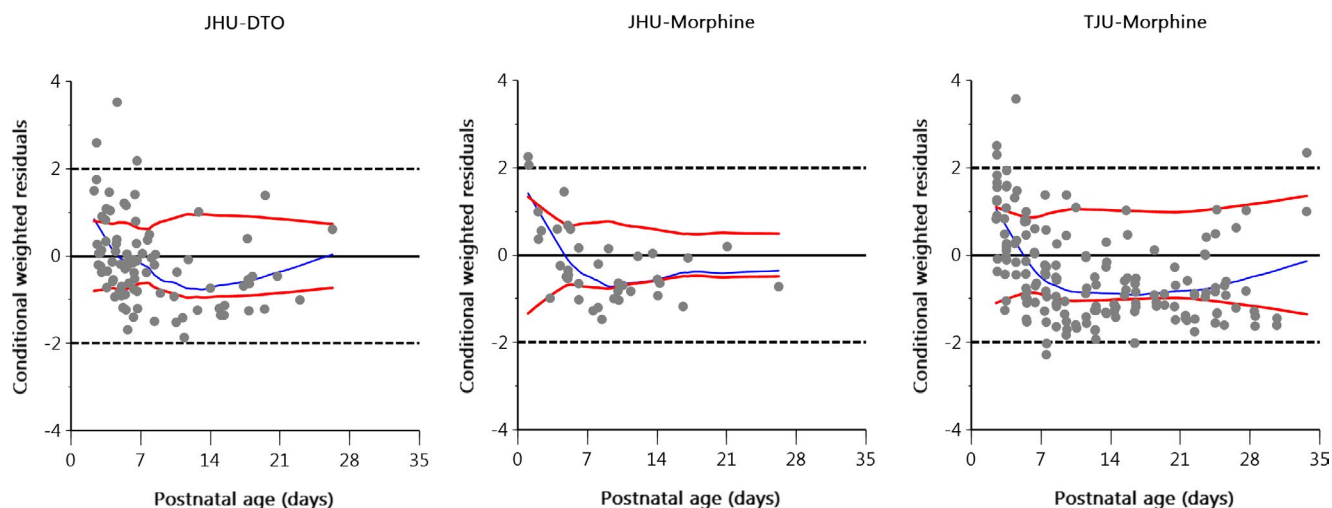
$$F = 1 - E \cdot PNA \text{ Effect} \quad (6)$$

where  $k_a$  is the first-order absorption rate constant,  $A_a$  is the amount of morphine in the absorption compartment,  $A_c$  is the amount in the central compartment,  $A_p$  and  $A_d$  are the amounts in the peripheral compartment and deep peripheral compartment,  $Q_{cp}$  is the intercompartmental CL between central and peripheral compartments,  $Q_{cd}$  is the intercompartment CL between the central and deep peripheral compartments, and  $E$  is the extraction ratio. PNA effect is a PNA-dependent latent variable and theoretically ranges from  $(1 - \Delta)$  to 1, where  $PNA_{50}$  is the time to 50% of the PNA effect maturation,  $\Delta$  is the relative CL difference after birth to the matured PNA effect level, and the parameter  $\gamma$  is the sigmoid shape factor. At the time of birth ( $t = 0$  hour), PNA effect =  $(1 - \Delta)$ . Our assumption is that the intrinsic CL of morphine is PNA dependent and in turn affects both CL and  $F$ . Therefore, the PNA effect was directly added to CL and  $F$  simultaneously. The detailed information pertaining to the model parameterization and statistical model is given in the Supplementary Material as Phoenix Modeling Language (PML) code.

### Model evaluation

Model evaluation was based on various goodness-of-fit indicators, including mechanistic plausibility, visual inspection of diagnostic scatter plots, and precision of estimates of population-fixed and random-effect parameters. Specifically, the conditional weighted residuals (CWRES) vs. PNA plot was used to evaluate the improvement in the model predictions.

The nonparametric bootstrap method was used to estimate the parameter precision and model stability. A total of 200 replications were generated by resampling from the observed morphine concentration data sets, and PK parameters were estimated for each of the replicated data sets separately. The median and corresponding 95% percentile interval (2.5th and 97.5th percentiles) obtained from the 200 sets of parameter estimates were compared with the estimates obtained from first-order conditional estimate with interaction.



**Figure 2** Diluted Tincture of Opium (DTO) model external evaluation in the Johns Hopkins University (JHU)-Morphine and Thomas Jefferson University (TJU)-Morphine trials: conditional weighted residuals vs. postnatal age. The red line and blue lines represent locally estimated scatterplot smoothing (LOESS) regression and the corresponding 95% confidence intervals.

Population PK model analysis was performed using Phoenix NLME 7 (Certara, L.P., St. Louis, MO). The first-order conditional estimate with interaction method was applied in the modeling process. All of the plots were generated in Phoenix.

## RESULTS

A total of 18 prospectively enrolled late preterm and full-term neonates from the JHU-Morphine study were included. The 18 neonates included 3 with ICU-acquired NAS and 15 with *in utero*-acquired NAS. These 18 neonates provided 51 plasma samples from which the parent drug morphine and the two major metabolites, morphine-3-glucuronide and morphine-6-glucuronide, were measured. On average, 2–4 plasma samples were collected for each neonate.

A total of 29 prospectively enrolled full-term neonates from TJU-Morphine study (also known as the Blinded Buprenorphine OR Neonatal Morphine Solution trial) were also included.<sup>15</sup> Of the 30 neonates in the original clinical trial, 1 was excluded because of incomplete information. All of the 29 neonates included acquired NAS *in utero* and together provided 209 plasma samples. On average, 7 samples were collected from each neonate with a minimum of 4 and a maximum of 16 samples.

Data from the 34 neonates with 88 morphine plasma samples after oral administration of DTO from our previously published clinical study in neonates were also included in the model development.<sup>11,12</sup> This was included to maximize the information content for model development.<sup>18</sup>

In total, 81 neonates from three clinical trials conducted at JHU and TJU were included. The descriptive statistics of their demographics and baseline characteristics are given in **Table 1**.

### Original DTO model

The previous DTO model was based on the intravenous model from the literature and JHU-DTO data.<sup>11</sup> The JHU and TJU

**Table 1** Patient characteristics at baseline in each clinical trial

Variable	JHU-DTO (N = 34)	JHU-Morphine (N = 18)	TJU-Morphine (N = 29)
PNA (day)	2.0 ± 0.90	2.71 ± 4.21	1.06 ± 2.49
PMA (week)	39.1 ± 2.1	38.2 ± 1.62	39.5 ± 1.10
WT (kg)	2.9 ± 0.4	2.79 ± 0.60	2.98 ± 0.40
<i>In utero</i> methadone	88.2%	83.3% <sup>a</sup>	93.1%
Breastfeeding	0%	0%	27.6%

Data are presented as mean ± standard deviation.

DTO, diluted tincture of opium; JHU, Johns Hopkins University; PNA, postnatal age; PMA, postmenstrual age; TJU, Thomas Jefferson University; WT, body weight.

<sup>a</sup>The other 16.7% were iatrogenic neonatal abstinence syndrome.

clinical trials were used to evaluate the DTO model initially. All of the model diagnostic plots were acceptable unless the diagnostic plot involved evaluation with PNA, e.g., the plot of CWRES vs. PNA.<sup>19</sup> The CWRES vs. PNA diagnostic plots are given in **Figure 2** by each clinical trial, including the one from the original DTO trial. This plot strongly suggested that the morphine plasma concentrations were underpredicted during the first week of life and overpredicted after the first week of life after evaluating the DTO model, although this phenomenon was not obvious when only the DTO clinical trial data were included (**Figure 2, left**). This PNA-related model bias was not found in the plot of CWRES vs. PMA. Also, the observed (red solid dots) and individual predicted (blue lines) concentration–PNA profiles in four representative patients for the DTO model external evaluation are shown in **Figure 3**. The same conclusion could be made based on the visual inspection of the concentration time profiles.

### Modified morphine model

A PNA-dependent latent variable, which represents the change of intrinsic CL, was successfully added to the DTO model to influence both the *F* (in Eq. 6) and systematic CL

(in Eq. 2) of morphine as shown in **Figure 1**. A lower baseline level of PNA effect was estimated as a fraction ( $1 - \Delta$ ) to account for the lower intrinsic CL at birth. The PNA effect reaches a value of 1 when the PNA-dependent intrinsic CL matures.

The final parameter estimates with bootstrap evaluation are given in **Table 2**. The plots of CWRES vs. PNA shown in **Figure 4** show no PNA-dependent bias in any of the three clinical trials using the modified model, and the improvement in the current model is obvious in comparison with the DTO model prediction in **Figure 2**. A comparison of concentration–time profiles between the previously published DTO model with the current model was given in **Figure 3**.

## DISCUSSION

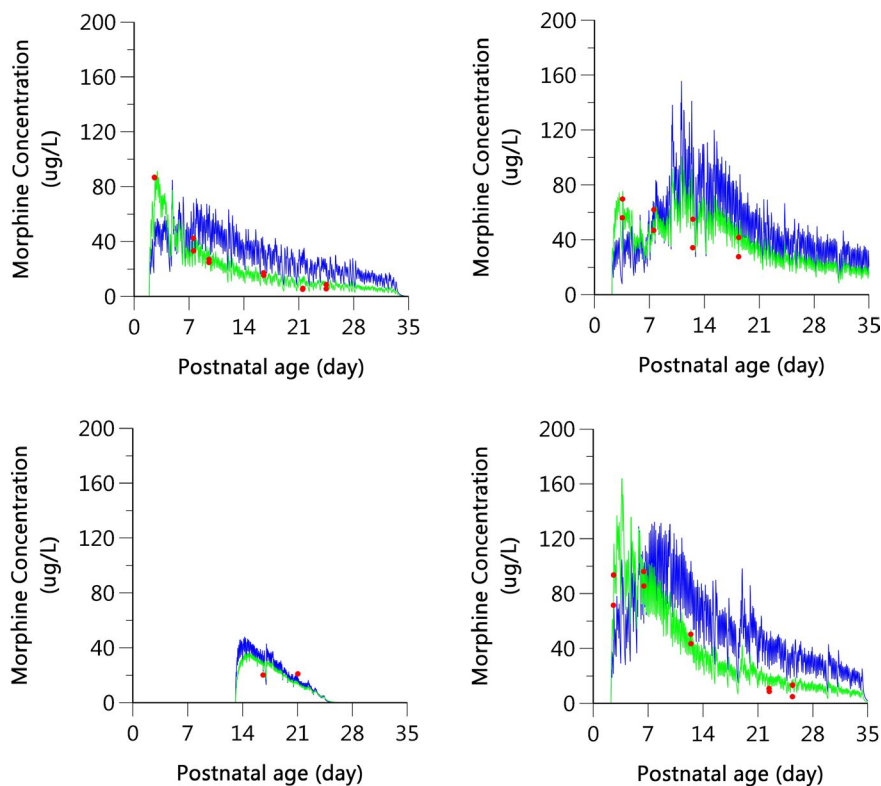
Models continuously evolve with our understanding of the underlying mechanisms. In this research, we improved the previously reported model by accounting for the PNA-dependent morphine metabolism in neonates.<sup>11</sup> The JHU-DTO trial collected about 2–3 samples per patient for 21 days. The availability of rich PK data from the JHU and particularly TJU populations permitted us to establish a consistent pattern.

In addition to our current findings of PNA effect on morphine CL, consistently lower glucuronidation activity during the first week of life has been reported. For instance, in another previously published morphine PK model in neonates

and infants, a 58% lower CL during the first 10 days of life was reported after adjusting for body size in 248 pediatric patients, including 64 preterm neonates and 59 term neonates.<sup>20</sup> Similarly, propofol is mainly metabolized by UGT1A9, and its CL was found to be 51% lower during the first 10 days of life after adjusting for body weight and PMA effect on CL in 25 neonates.<sup>21,22</sup> Furthermore, the zidovudine, which is also mainly metabolized by UGT2B7, CL has been reported to be doubled in neonates after 7 days of life when compared with the first day of life in 16 neonates.<sup>23–25</sup>

Enzyme maturation is typically believed to be correlated with PMA.<sup>26</sup> However, the consistent lower glucuronidation activity during the first week of life across different UGT families and different substrates suggested a common phenomenon in neonatal glucuronidation activity that is correlated with PNA. The reason for lower glucuronidation activity does not seem to be a result of enzyme maturation, which is always correlated with PMA, especially when the lower glucuronidation activity was reported after the adjustment for body weight and PMA. Consequently, the following question was raised: what are the potential contributing factors to the consistently observed PNA effect on glucuronidation activity?

Uridine diphosphate glucuronic acid (UDPGA) concentration-dependent glucuronidation activity in human hepatocytes has been reported based on an *in vitro* study.<sup>27</sup> Also, fasting reduces UDPGA content in rats and results in a decreased bilirubin glucuronidation rate.<sup>28</sup> The depletion of hepatic UDPGA by UGT substrates suggested that



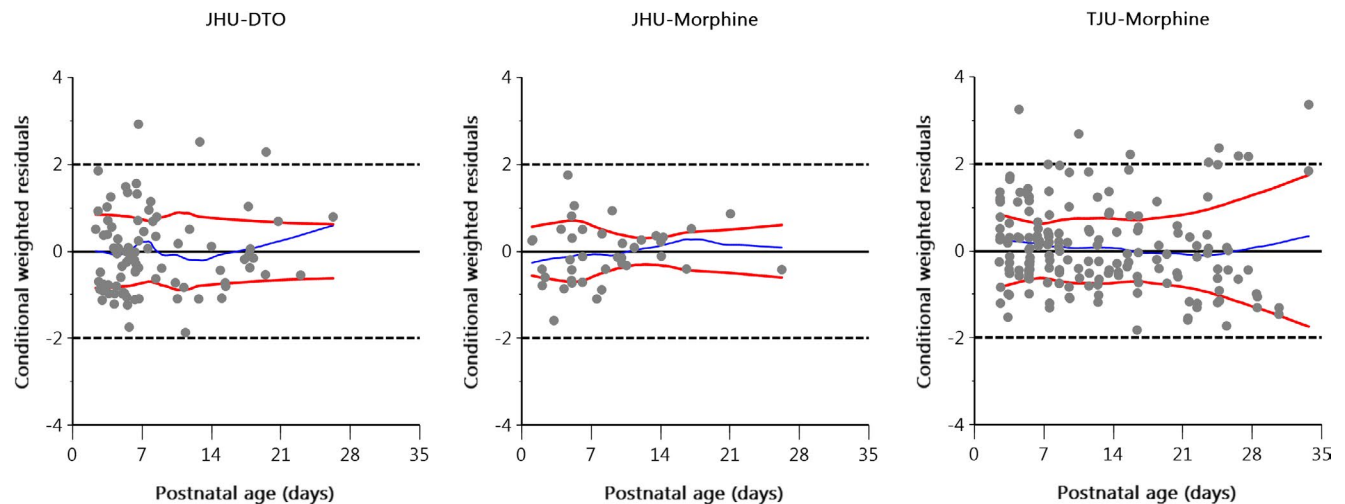
**Figure 3** Individual predicted morphine concentration–time profile in four representative subjects for model external evaluation (red solid dots = observed morphine concentration, blue line = Diluted Tincture of Opium (DTO) model predicted morphine concentration, green line = current model predicted morphine concentration).

**Table 2** Final parameter estimates

	FOCE-I		Bootstrap	
	Estimate 95% CI	BSV	Median 95% PI	BSV
$K_a$ (1/hour)	0.666 (0.419–0.913)	–	0.709 (0.195–1.176)	–
$E$	0.808 (0.778–0.838)	–	0.804 (0.771–0.837)	–
$V_{c, \text{std}}$ (L)	17.8 <sup>a</sup>	–	17.8 <sup>a</sup>	–
CLstd (L/hour)	75.3 <sup>a</sup>	30.2% (20.3–37.6%) <sup>b</sup>	75.3 <sup>a</sup>	29.2% (10.0–40.1%) <sup>b</sup>
$V_{p, \text{std}}$ (L)	87.3 <sup>a</sup>	–	87.3 <sup>a</sup>	–
$Q_{cp, \text{std}}$ (L/hour)	136 <sup>a</sup>	–	136 <sup>a</sup>	–
$V_{d, \text{std}}$ (L)	199 <sup>a</sup>	–	199 <sup>a</sup>	–
$Q_{cd, \text{std}}$ (L/hour)	19.5 <sup>a</sup>	–	19.5 <sup>a</sup>	–
CLmat <sub>50</sub> (week)	58.3 <sup>a</sup>	–	58.3 <sup>a</sup>	–
HillCL	3.6 <sup>a</sup>	–	3.6 <sup>a</sup>	–
$\beta_{\text{Vol}}$	0.614 <sup>a</sup>	–	0.614 <sup>a</sup>	–
$T_{\text{Vol}}$ (year)	0.185 <sup>a</sup>	–	0.185 <sup>a</sup>	–
PNA <sub>50</sub> (day)	2.88 (1.56–4.19)	50.6% (28.3–65.7%) <sup>b</sup>	2.87 (1.92–4.09)	48.9% (27.6–62.9%) <sup>b</sup>
$\Delta$	0.660 (0.389–0.932)	–	0.668 (0.441–0.884)	–
$\gamma$	2.21 (1.24–3.19)	–	2.32 (1.57–3.53)	–
Proportional residual errors	0.392 (0.352–0.432)	–	0.389 (0.337–0.436)	–

$\Delta$ , relative UDPGA difference after birth to matured uridine diphosphate glucuronic acid level;  $\gamma$ , a sigmoid-shape factor; BSV, between-subject variability;  $\beta_{\text{Vol}}$ , fractional difference from  $V_{c, \text{std}}$  at birth; CI, confidence interval; CL, clearance; CLmat<sub>50</sub>, the postmenstrual age at which CL was 50% of the mature adult value; CLstd, CL for the body weight of 70 kg;  $E$ , extraction ratio (in PNA  $\geq 7$  days); FOCE-I, first-order conditional estimate with interaction; HillCL, the Hill coefficient for CL that defines the steepness of the maturation curve;  $k_a$ , absorption rate constant; PI, percentile interval; PNA, postnatal age; PNA<sub>50</sub>, postnatal age at uridine diphosphate glucuronic acid level at 50% of maturation;  $Q_{cd, \text{std}}$ , intercompartment CL between the central compartment and deep peripheral compartment for the body weight of 70 kg;  $Q_{cp, \text{std}}$ , intercompartment CL between central compartment and peripheral compartment for the body weight of 70 kg;  $T_{\text{Vol}}$ , maturation half-life of the PNA-related changes of  $V_c$ ; UDPGA, uridine diphosphate glucuronic acid;  $V_{d, \text{std}}$ , deep peripheral compartment volume of distribution for the body weight of 70 kg;  $V_{c, \text{std}}$ , central volume of distribution for the body weight of 70 kg;  $V_{p, \text{std}}$ , peripheral compartment volume of distribution for the body weight of 70 kg.

<sup>a</sup>Parameters were fixed as in the previous publication<sup>9</sup>. <sup>b</sup>The CI of BSV on CL was calculated based on the BSV on CL and the corresponding standard error (SE) assuming  $t$  distribution.



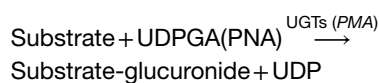
**Figure 4** Model with postnatal age as prognostic factor in the Johns Hopkins University (JHU)-Morphine and Thomas Jefferson University (TJU)-Morphine trials: Conditional weighted residuals vs. PNA. The redline and blue lines represent locally estimated scatterplot smoothing (LOESS) regression and the corresponding 95% confidence intervals. DTO, diluted tincture of opium.

the metabolism of UGT substrates could further reduce the UDPGA in animals.<sup>29</sup> More important, the use of phenobarbital for UGT1A1 induction in neonates was shown to be incapable of increasing the CL of endogenous UGT1A1 substrate bilirubin in the first 4 days after birth, and the lower UDPGA level

was shown to be responsible for the hyperbilirubinemia in neonates during the first week of life.<sup>30</sup> Therefore, historical data from *in vitro*, animal, and human studies all suggested that UDPGA could be a rate-limiting factor in glucuronidation activity. Furthermore, in the TJU-Morphine trial, five neonates were

exposed to phenobarbital, but they did not show any difference in their morphine PK, which may also indicate UDPGA-limited, but not UGT2B7-limited, glucuronidation activity.

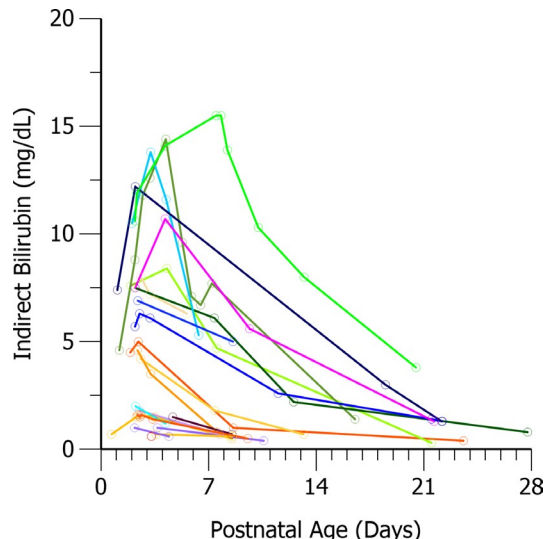
UDPGA is made from uridine diphosphate glucose by uridine diphosphate glucose 6-dehydrogenase, and the uridine diphosphate glucose is synthesized by uridyl transferase from glucose-1-phosphate and uridine triphosphate.<sup>31,32</sup> The increasing plasma glucose level during the first week of life<sup>33</sup> may result in an increasing UDPGA concentration. Therefore, the formation of UDPGA could be a function of PNA and lead to PNA-dependent glucuronidation activity and hence morphine metabolism in the first week of life. Under the hypothesis that UDPGA activity reflects PNA-dependent changes in glucuronidation in addition to the previously shown PMA effect, the mechanistic explanation of PMA and PNA effect on glucuronidation in neonates could be summarized in the following formula:



where the concentration of UDPGA is a function of PNA and the UGT enzyme expression is a function of PMA. During the first week of life, the lower concentration of UDPGA may be the rate-limiting step for glucuronidation activities. Afterward, substantial UDPGA is available at the site of action, and the UGT levels, which are dependent on PMA, are the rate-limiting factors.

Mechanistic support for the role of PNA-dependent UDPGA activity can also be gleaned from bilirubin, which is mainly metabolized by UGT1A1.<sup>34</sup> Bilirubin is part of the standard hepatic function evaluation in newborns. The concentration of bilirubin increases until 3–4 days after birth (i.e., slower bilirubin CL) and then decreases (i.e., faster CL). The individual bilirubin concentration–time profiles from the 29 neonates in the TJU-Morphine study are depicted in **Figure 5**. Motivated by similar conceptualization, Smits *et al.*<sup>35</sup> surmised a relationship between hyperbilirubinemia and propofol CL. However, hyperbilirubinemia (bilirubin concentration elevated or not at any point) failed to show a significant effect on propofol CL. Bouwmeester *et al.*<sup>10</sup> found that bilirubin concentration is a predictor of morphine CL in the formation of morphine-3-glucuronide, perhaps as a generic measure of hepatic function. Unlike these reports that employed only a time-independent bilirubin measure, in our opinion, the rate of change of bilirubin that reflects a change in metabolic activity over age is a better metric. A higher bilirubin concentration does not necessarily indicate a lower bilirubin elimination rate, but it could also be possible because of a higher bilirubin production rate. Therefore, it is not bilirubin concentration but the rate of change of bilirubin concentration that likely correlates with morphine CL.

Gathering support from *in vitro* and *in vivo* evidence, we propose that the previously reported bilirubin effect on morphine CL by Bouwmeester *et al.*<sup>10</sup> is not only a general covariate for hepatic function but also mechanistically a result of lower UDPGA concentration. In the correlation with bilirubin, the CL of morphine and propofol may not be a function



**Figure 5** Observed individual indirect bilirubin concentration vs. postnatal age in the 29 neonates from the Thomas Jefferson University (TJU) trial.

of the bilirubin concentration or hyperbilirubinemia, but to the change of bilirubin elimination rate.

Correlation is expected between PMA and PNA in a population setting. However, this correlation between PNA and PMA in the context of morphine-based pharmacotherapy in NAS needs to be understood differently, as explained here. Normally, the treatment of NAS takes 2–4 weeks. Consider two neonates: neonate 1 and neonate 2. Neonate 1 was delivered at a gestational age of 34 weeks followed by 3 weeks of morphine therapy. Upon completion of the treatment, neonate 1's PNA is 3 weeks and PMA is 37 weeks. Neonate 2 was delivered at a gestational age of 38 weeks followed by 3 weeks of morphine therapy. Upon completion of the treatment, neonate 2's PNA is 3 weeks, but the PMA is 41 weeks. Although both neonates have identical PNA, their PMAs are different. Therefore, PMA and PNA are not truly correlated in our neonatal population with respect to this pharmacotherapy.

In this study, the gestational age ranged from 32–40 weeks. With the 2–4 weeks of exposure to morphine therapy in these NAS patients, gestational age played a major role as part of the PMA calculation, and the PMA for those neonates with abstinence syndrome is not highly correlated with PNA as described previously. The biased trend over PNA observed in **Figure 2** for the JHU-DTO data could not be replicated when using PMA. A mechanistic reason for the relevance of PNA over PMA to describe UDPGA activity has yet to be explored, but the empirical evidence seems to be strong.

Because of the lack of morphine concentration data after intravenous administration in these patients, we cannot independently evaluate the PNA effect on systemic CL. Hence, PNA was assumed to have the same magnitude of impact on both *F* and CL. This makes mechanistic sense, as the presystemic CL mechanism is similar to systemic.

A note on the *F* estimate is warranted. We previously estimated the *F* of oral morphine to be 54%.<sup>11</sup> We now

appreciate that during the initial days after birth, the CL is low and hence the  $F$  is higher and vice-versa. As the plasma concentrations in the JHU-DTO trial were predominantly collected during the first 2 weeks after birth, the  $F$  was estimated to be 54%. In patients older than 7 days (PNA), the  $F$  based on our current model is 20% ( $F = 1 - E$ ), as the CL is higher in these patients, which is consistent with the previously reported  $F$  of 23.8% in adults.<sup>36</sup>

Using an *in vitro* study, Morrish *et al.*<sup>37</sup> reported a methadone inhibition effect on morphine metabolism. A majority of the neonates in our study were exposed to methadone *in utero* before their delivery (Table 1). This could be postulated as a reason for the lower metabolism of morphine during the first week of life because of the potential for drug interaction. However, a comparison between the methadone-exposed and non-methadone-exposed neonates did not show meaningful differences on the PK parameters. Furthermore, after birth the withdrawal symptoms manifest and become severe only after this *in utero* methadone exposure is diminished from the neonate. This inference was based on evidence from one of the three clinical trials in this study in which urine for an opiate test was collected from the neonates right after birth. Of these neonates, 35% were opiate positive. It usually takes 2 days for a neonate with NAS to experience severe withdrawal symptoms that require pharmacotherapy. Given that methadone half-life in neonates is 4–6 hours, in 48 hours (8 half-lives), the methadone levels should have been completely eliminated. This subsequently triggers pharmacotherapy with morphine as a result of the worsening of symptoms. In other words, the initiation of morphine therapy indicated a lack of methadone exposure in the systemic circulation, and hence the potential for drug interaction via methadone inhibition on morphine metabolism is unlikely.

We have chosen to fix the allometric exponents based on the strong principles of clinical pharmacology,<sup>26</sup> although there are other schools of thought that advocate for the estimation of such exponents. Recently, the European Medical Agency put out a note for analyzing pediatric data. The European Medical Agency suggests that fixing to theoretical values (e.g., 0.75) is preferred.<sup>38</sup> As part of the sensitivity analysis, we also estimated the exponent for comparison to the fixed approach. The estimate of the exponent on the body weight effect on CL suggested a value of 0.90, which was close to the fixed exponent of 0.75 in our final model. Also, the estimate of the exponent on the body-weight effect on volume of distribution was 0.95, which was also close to the fixed exponent of 1 in our model.

In the JHU-Morphine study, two neonates experienced ICU-acquired NAS. The PK parameters of these two neonates were similar to those for the *in utero*-acquired NAS neonates. However, because of the limited number of ICU-acquired NAS neonates, this comparison was not conclusive.

In this research, we proposed a new hypothesis for the PNA-dependent lower glucuronidation activity in the first week of life and linked the relationship between bilirubin and the CL of morphine by the UDPGA levels after birth. The hypothesis was extended beyond the prediction

of morphine CL to all glucuronidation activity in the first week of life. The role of UDPGA levels after birth in the CL of morphine is perhaps one of many potential reasons for the lower glucuronidation. Because this is a retrospective analysis to characterize morphine PK in neonates, bilirubin samples were not collected in all of the neonates, and UDPGA measurements were not collected. Further experimental data, especially UDPGA concentrations, may be required to prove this hypothesis. However, the *in vitro* and *in vivo* evidence from the literature supports the findings in its current form and provides a hypothesis for future research to be conducted.

**Supporting Information.** Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website ([www.psp-journal.com](http://www.psp-journal.com)).

#### PML Code.

**Acknowledgment.** The authors would like to thank all of the patients, nurses, and physicians who were part of the clinical trials.

**Funding.** The Diluted Tincture of Opium (DTO) study was funded by a Thomas Wilson grant and an institutional research grant from Johns Hopkins Hospital, General Clinical Research Center and was supported by National Institute on Drug Abuse Grant IR21DA016288. In addition, the Clinical Pharmacology National Institutes of Health Training Grant 5T32GM066691 supported Dr Tamorah Lewis during this work. Dr Sartori was supported by an Education Grant from Thermo Fisher Scientific. The Thomas Jefferson University (TJU)-Morphine/Blinded Buprenorphine OR Neonatal Morphine Solution trial was supported by a grant (R01DA029076) from the National Institute on Drug Abuse. Jason Moore was supported by Clinical Pharmacology National Institutes of Health Training Grant T32GM008562.

**Conflict of Interest.** The authors declared no competing interests for this work.

**Author Contributions.** T.L., T.R.L., J.G., W.K., and V.I. wrote the manuscript. T.R.L., E.G., and W.K. designed the research. T.R.L., E.G., W.K., D.S., J.M., and D.M. performed the research. T.L., T.R.L., J.G., and V.I. analyzed the data.

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