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ABCC3 and OCT1 genotypes influence pharmacokinetics of morphine in children

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Abstract

Aim—Large interindividual variability in morphine pharmacokinetics could contribute to variability in morphine analgesia and adverse events.

Methods—Influence of weight, genetic polymorphisms, race and sex on morphine clearance and metabolite formation from 220 children undergoing outpatient adenotonsillectomy was studied. A nonlinear mixed effects model was developed in NONMEM to describe morphine and morphine glucuronide pharmacokinetics.

Results—Children with *ABCC3* -211C>T polymorphism C/C genotype had significantly higher levels of morphine-6-glucuronide and morphine-3-glucuronide formation (~40%) than C/T+T/T genotypes ($p < 0.05$). In this extended cohort similar to our earlier report, *OCT1* homozygous genotypes ($n = 13$, *OCT1* *2-*/5/*2-*/5) had lower morphine clearance (14%; $p = 0.06$), and in addition complementing lower metabolite formation (~39%) was observed. *ABCB1* 3435C>T TT genotype children had lower levels of morphine-3-glucuronide formation though no effect was observed on morphine and morphine-6-glucuronide pharmacokinetics.

Conclusion—Our data suggest that besides bodyweight, *OCT1* and *ABCC3* genotypes play a significant role in the pharmacokinetics of intravenous morphine and its metabolites in children.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Keywords

ABCC3; ABCB1; morphine; OCT1; pediatrics; pharmacogenetics; population pharmacokinetics; surgical pain

Background

Morphine is a commonly used opioid to manage severe pain after surgery. Large between patient variability in morphine pharmacokinetics (PK) may contribute to the unpredictable variability in morphine analgesia and adverse effects, especially in children. African–American children have inadequate surgical pain relief while Caucasian children have more adverse events with similar doses of intravenous morphine [1]. Moreover, African–American children have significantly higher morphine clearance (CL) than children of Caucasian descent [2]. We believe that racial differences observed in analgesic response could in part be explained by the underlying racial differences in morphine PK. We recently demonstrated that variants of the *OCT1* gene were associated with differential hepatic morphine uptake and PK of intravenous morphine, which partly explains racial differences in morphine CL [3].

The analgesic response and adverse effects observed post morphine dose are a result of the combined pharmacological effects of morphine and its metabolites. Variations in PK of morphine may contribute to interindividual differences in response to opioids such as morphine. Morphine's PK variations can be examined through genetic polymorphisms that alter the functionality of key enzymes and membrane transporters that impact its metabolism and tissue distribution. Morphine is metabolized by a variety of pathways with approximately 70% of morphine converted by glucuronidation to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) [4]. Morphine metabolic elimination occurs primarily in the liver and is mainly catalyzed by UGT2B7. The UGT2B7 –161C>T genotype was shown to have reduced morphine metabolic ratios (M6G/morphine) with an increasing number of T alleles [5]. M6G is considered more potent than morphine, and its analgesic activity is mediated like morphine through opioid receptors. M3G, on the other hand is responsible for the partial antagonism of morphine and M6G-induced analgesia [6].

A better understanding of PK of morphine along with its metabolites could help better delineate the observed high variability in analgesic response. A range of transporters including OCT1, ABCB1, ABCC2 and ABCC3 have been known to play a significant role in the disposition of morphine and its metabolites based on in mice and other *in vitro* studies [4,7-14] (Figure 1). Tzvetkov *et al.* clearly show morphine to be an OCT1 substrate and further demonstrated *OCT1* poly morphisms that resulted in impaired activity and that impact on morphine uptake *in vitro* [11]; these findings are supportive of and validate our associations in children receiving morphine. An efflux transporter expressed in the basolateral membranes of hepatocytes, ABCC3, is known to efflux M3G and M6G into the bloodstream. The mRNA expression in the liver tissue was found to be lower in subjects with the –211C>T TT genotype, which might potentially contribute to a lower morphine efflux of morphine glucuronides [15,16]. ABCC2, expressed in the canalicular side, transports morphine glucuronides in mice into bile [7,12], while morphine is not known to

be a substrate. *ABCC2* genotypes 1249G>A and 3972C>T have been associated with altered CL for carbamazepine [17] and talinolol [18], though their effect on morphine disposition is unknown. Another study found that a subject with the *ABCB1* 3435C>T homozygous genotype had a high maximum cerebrospinal fluid (CSF) concentration of morphine. The *ABCB1* 3435C>T allele has also been linked with higher morphine analgesia in cancer-related pain and lower morphine dose requirements in a mixed chronic pain population [19,20]. However, the role of *ABCB1* in morphine PK is not well known.

To address the current knowledge gap in the role of genetic variations on morphine PK, we hypothesized that common functionally defective genetic polymorphisms of genes coding for key transporters and enzymes (including OCT1, *ABCC3*, *ABCB1*, *ABCC2* and *UGT2B7*) can substantially alter the PK of morphine and its metabolites. The aim of this prospective clinical study was to evaluate the potential impact of selected genetic variants of key transporters and enzymes on intravenous morphine PK in an extended homogeneous cohort of children undergoing tonsillectomy.

Methods

Study design

This study is a part of an ongoing clinical study entitled 'Personalizing Perioperative Morphine Analgesia in Children' registered with clinicaltrials.gov (NCT01140724). This is a prospective, genotype-blinded study in a large cohort of children undergoing outpatient adenotonsillectomy receiving standard perioperative care to identify factors predictive of interindividual variability in analgesic response and adverse effects to perioperative opioids. Informed consent and assent from parents and children, and approval from the institutional review board at Cincinnati Children's Hospital Medical Center (OH, USA) were obtained before enrollment.

Participants

Children aged 6–15 years, with an American Society of Anesthesiologists physical status of 1 or 2 scheduled for adenotonsillectomy because of recurrent tonsillitis, adenotonsillar hypertrophy or obstructive sleep apnea were enrolled. Children having morphine allergy, developmental delay, hepatic/renal diseases, chronic analgesic requirement and children or parents unable to speak English were excluded [1-3]. Patients received standard perioperative care along with one intraoperative intravenous morphine bolus dose of 0.2 mg/kg. Children with obstructive sleep apnea received a morphine dose of 0.1 mg/kg.

PK sampling

Serial blood samples were obtained from an individual child to quantify morphine, M3G and M6G systemic concentration. A predose sample was obtained before intravenous morphine bolus dose from an intravenous line. Further samples were obtained using independent venous needle sticks 0–5, 10–15 and 30–45 min after the first bolus morphine intravenous dose. For ethical reasons, minimal number of blood samples (1–3) were obtained before the child fully recovered from anesthesia in the recovery room.

PK analysis

Morphine and its active metabolites, M3G and M6G, were quantified in EDTA plasma using a validated liquid chromatography tandem mass spectrometry assay. Details of the analytical methods have been described elsewhere [21]. The reliable limits of quantification were 0.25–1000 ng/ml ($r^2 > 0.99$) for morphine, and 1–1000 ng/ml ($r^2 > 0.99$) for both M3G and M6G. Total imprecision was less than 15%. The interday accuracy was within 85–115%. There was no carry over, matrix interferences or ion suppression/enhancement interfering with the quantification of the analytes.

Genotyping

Blood was collected in the operating room using an intravenous line placed for anesthesia; this blood was used for genotyping relevant polymorphisms. DNA was isolated and tested for specific SNP within the preselected list of functionally important genes (*OCT1*, *ABCC3*, *ABCB1* and *UGT2B7*). Commercially available TaqMan® SNP genotyping assays and Illumina (CA, USA) human Omni 5 Genome-Wide Human Array were used for genotyping. Please refer to Supplementary Table 1 (see online at: www.futuremedicine.com/doi/suppl/10.2217/pgs.14.99) for a consolidated list of all the SNPs genotyped, their source of genotyping and results of the appropriate tests used for quality control. The participants were genotyped for four nonsynonymous SNPs in the *OCT1* gene, which cause reduction or loss of OCT1 transporter activity, including Arg61Cys (rs12208357), Gly401Ser (rs34130495), Gly465Arg (rs34059508) and the deletion of Met420 (rs72552763) [3]. Subjects were divided into three groups based on the *OCT1* genotype as described earlier [3]. In addition, rs4793665 (–211C>T) a SNP in the promoter region of *ABCC3* that is known to alter mRNA expression was genotyped. *ABCC2* genotype for SNPs 1249G>A (rs2273697) and 3972C>T (rs3740066) were determined as they have been associated with altered PK of carbamazepine [17] and talinolol [18]. The *ABCB1* 3435C>T genotype was included as it has been repeatedly associated with dose requirement and was associated with higher morphine concentration in CSF [19,22,23]. The *UGT2B7* –161C>T genotype (known to be in complete linkage disequilibrium with 802T>C) was also included as earlier reports have shown morphine metabolic ratios (M6G/morphine) reduced with an increasing number of T alleles [5].

Morphine PK model development & evaluation

A population PK model was developed for morphine using a nonlinear, mixed effects modeling approach (NONMEM; version 7.2, ICON Development Solutions, MD, USA) with PsN-Toolkit (version 3.5.3) as the interface. Data preprocessing, postprocessing and visualization were performed using the statistical package R (version 2.15). A two compartment structural model, parameterized in terms of CL, central volume of distribution (V_1), intercompartmental CL (Q) and peripheral volume (V_2) of distribution was used to describe the morphine concentration–time profiles (Figure 2). A delay compartment was incorporated in the model to describe the delay metabolite formation. The metabolite PK was modeled using an additional compartment for each metabolite and was parameterized in terms of formation CL (FCL; FCL_{M3G} and FCL_{M6G}), volume of distribution (V_{M3G} and V_{M6G}) and CL (CL_{M3G} and CL_{M6G}). Incorporation of delay and metabolite compartments

did not alter the morphine mass balance. A more detailed discussion of model equations and model development is presented in the Supplementary Section 1.

The effect of body size on the PK parameters was normalized using allometric scaling, which has a strong theoretical and empirical basis [24] and has been recommended for use for pediatric PK analysis [25]. Weight exponents of 0.75 and 1.0 were used to normalize the CLs and volumes of distribution, respectively:

$$CL_{mean,WT} = CL_{popmean,70Kg} \left(\frac{WT}{70} \right)^{0.75}$$

$$V_{mean,WT} = V_{popmean,70Kg} \left(\frac{WT}{70} \right)^{1.0}$$

where $CL_{mean,WT}$ and $V_{mean,WT}$ are population mean CL and volume for a child's weight (WT) in kg. Between subject variability was described by an log-normal parameter distribution. A proportional error model was used to account for unexplained residual variability with σ_M^2 , σ_{M3G}^2 and σ_{M6G}^2 representing the variances for morphine, M3G and M6G, respectively.

Initial comparison between different models was based on a drop in objective function value (OFV). A drop in OFV of 3.84 ($p < 0.05$, degrees of freedom = 1) between nested models was considered statistically significant. In addition to OFV improvement, models were evaluated using diagnostic goodness-of-fit plots examined to identify possible trends suggestive of model mis-specification, η -distribution histograms we examined to ensure unimodality. The final model stability was evaluated by refitting the model to 1000 randomly sampled bootstrap datasets.

Pharmacogenetic analysis

Covariate analysis was performed to test selected genotypes as predictive covariates for individual *post hoc* Bayesian estimates of weight normalized morphine CL, M3G and M6G FCLs. Preliminary pharmacogenetic (PG)–PK analysis was carried out using Fisher's one-way analysis of variance with $p < 0.05$ considered statistically significant. Bonferroni correction was used for multiple testing when multiple inheritance models were tested. The significant covariates were then tested in NONMEM by incorporating the genotype as a categorical covariate for morphine CL and metabolite FCLs. The significance of a genotype as a covariate in morphine PK was determined using a twofold criteria: a decrease in the OFV of 3.84 ($p < 0.05$, degrees of freedom = 1) between nested models was considered as a significant covariate for the PK model; and whether the precision of the covariate parameter estimates were significantly ($p < 0.05$) different than 0.

Results

Demographics

Detailed demographics of the enrolled subjects are presented in Table 1. Of the 220 enrolled subjects, 176 were Caucasian, 38 were African–American while the remaining six subjects included one child of Asian descent, one child of Asian Indian decent and four biracial children with one Caucasian and one African–American parent. No significant differences in age, weight, height and male/female ratio were observed between Caucasian and African–American children (Table 1).

Distribution of genotypes

Distribution of SNP genotypes for the selected genes is summarized in Tables 2 & 3 (please also refer to Supplementary Tables 2 & 3). Based on the genotyping results, 58% of children were *OCT1* wild-type, 36% were *OCT1* heterozygous while only 5.4% were *OCT1* homozygous. Of the 13 homozygous children, 12 were Caucasian, one was Hispanic though none were of African–American descent. Racial heterogeneity was observed for the 3435C>T genotype in *ABCB1*, with 34.6% of Caucasians and only 5.3% of African–Americans having the TT polymorphism. ABCC3 –211C>T genotype CC was observed in 33% of our population and there was no difference in allelic frequency between both races.

Morphine & metabolite PK & modeling

A total of 711 plasma samples were collected from the 220 enrolled subjects, of which 220 samples collected prior to initial morphine dose were not included in the final PK analysis. After internal quality inspection, M6G levels from the 178 subjects, M3G levels from 168 subjects and morphine levels from 220 subjects were included in the final PK analysis. Concentration–time profiles for morphine, M3G and M6G from the final PK dataset are displayed in Figure 3. For M3G, only 340 (69%) samples had concentrations above the limit of quantification while for M6G only 160 (33%) samples had concentrations above the limit of quantification. Consistent with our earlier report [2], early time samples (0–5 min) had M3G and M6G below the limit of quantification for 40.4 and 89.7% of the samples, respectively (Figure 3).

The PK of morphine was described using a two compartment model with interindividual variability on its systemic CL (Table 4; please see the Supplementary Sections 1-2, Supplementary Table 4 & Supplementary Figures 1-3 for more detailed results on PK model development). The metabolite profiles were captured using a single compartment model with interindividual variability on the FCLs (FCL_{M3G} and FCL_{M6G}). The volume of distribution and CL for the metabolites were fixed based on prior reports [26-28] as the profiles only contain information about their formation owing to short sampling duration.

The formation of metabolite follows first order kinetics and was modeled to be proportional to morphine concentrations in a hypothetical compartment, which lagged the central compartment; consequently resulting in a delay in metabolite formation (Figure 2 & Table 4). Addition of a delay compartment resulted in the removal of the over prediction of metabolite levels at earlier times (Supplementary Figures 2 & 3) and under predictions at

latter times and resulted in a significantly better model (difference in OFV [dOFV] = -343). Given the wide weight range (Table 1) in our pediatric population, allometric scaling was used to account for the effect of body size on PK parameters. Incorporation of allometric scaling on all CLs and volumes with exponents significantly improved model fits (dOFV = -182) [28,29]. The η -shrinkage on morphine CL and M3G FCL was low (14.6 and 17.5%, respectively) while it was higher (29.9%) for M6G FCL indicating limited information pertaining to M6G formation in this dataset [30]. Parameters for the final model with delay compartment and allometric scaling included are presented in Table 4 and were used for further PG analysis.

Role of *OCT1*

Visual inspection of the distribution of individual morphine CLs (Figure 4) suggests that homozygous *OCT* genotype subjects had lower mean CL and FCL_{M3G} than the wild-type and heterozygous group combined. Preliminary covariate analysis performed using one-way ANOVA found that the *OCT1* heterozygous group had lower *post hoc* Bayesian estimates of weight normalized CL ($p = 0.06$) and FCL_{M3G} ($p < 0.05$; Table 5). Consistently, *OCT1* genotype was a significant covariate for FCL_{M3G} (dOFV = -6.5; Table 5) based on NONMEM covariate analysis, with homozygotes having 39% lower CL than others. Based on the NONMEM analysis, *OCT1* showed a clear trend towards significance (dOFV = -3.5; Table 5) with the homozygous group having lower morphine CL (14%; 95% CI: 2–26%). A similar trend was observed for M6G FCLs, where the *OCT1* homozygous group had approximately 25% lower values than others, although these results were not statistically significant.

Role of *ABCC3*

Progressively higher metabolite FCLs and morphine CLs were observed in subjects with an increasing number of C alleles during a visual inspection of the individual CL with respect to *ABCC3* -211C>T genotype (Figure 4). Both recessive and dominant inheritance models have been used in *ABCC3* -211C>T genotype association [15,31]. One-way ANOVA analysis conducted using two alternative models (recessive [TT+CT vs CC] and dominant [TT vs CT+CC]) suggested that the evidence for the recessive inheritance model (one-way ANOVA: $p < 0.025$) was more statistically significant than the latter (one-way ANOVA: $p > 0.1$) across tests (data not shown). *ABCC3* genotype was found to be a significant covariate for FCL_{M6G} based on one-way ANOVA ($p = 0.03$; Table 5) and covariate inclusion in the NONMEM model (dOFV = -4.86; Table 5). Similarly, CC genotype had significantly higher FCL_{M3G} and its inclusion significantly improved the model fit (dOFV = -9) with CC genotype having 46% (95% CI: 4.3–88%) higher FCL_{M3G} than the TT and CT genotypes combined. Estimates for the increase in FCL_{M6G} were similar to that in FCL_{M3G}, although precision on the former parameter was lower. While the evidence of homozygous CC genotype having higher metabolite formation was reasonably clear, its effect on morphine CL was less pronounced. Inclusion of the *ABCC3* genotype as a covariate improved the model fit (dOFV = -1.9; Table 5), although is not significant. The CC genotype is estimated to have a moderately higher (8.1%; 95% CI: -0.7–16.8%) morphine CL than others.

Role of *ABCB1*

No discernable influence of the *ABCB1* 3435C>T genotype on CL and FCL_{M6G} was observed, although subjects with CT and CC genotype combined seemed to have higher FCL_{M3G} than subjects with the TT genotype (Figure 4). These trends were confirmed to be statistically significant based on one-way ANOVA analysis, which revealed that CT+CC genotype had an 18.4% higher FCL_{M3G} than TT genotype ($p = 0.04$; Table 5) while its effect on CL was minimal (1%). Consistent results were obtained from NONMEM analysis where 3435C>T genotype was a significant covariate for FCL_{M3G} (dOFV = -4.32 ; Table 5), but not for CL and FCL_{M6G}.

Role of *UGT2B7* & *ABCC2*

UGT2B7 –161C>T genotype was found to have little to no impact on morphine CL or formation of M3G and M6G (Supplementary Figure 4; one-way ANOVA test: $p > 0.05$). Similarly, *ABCC2* 1249G>A and 3972C>T genotypes did not alter morphine CL or metabolite formation (Supplementary Figures 5 & 6; one-way ANOVA test: $p > 0.05$).

Role of race & sex

Inclusion of race and sex as covariates of morphine CL tended to improve model performance, although the effect of race and sex was not found to be significant (dOFV = -2.4 and -3 ; Supplementary Table 4). Compared to African–American children, Caucasian children had approximately 7% less morphine CL. Compared to boys, girls had approximately 5% less morphine CL.

Discussion

Our large pediatric morphine PK and PG study offers further evidence that *OCT1* homozygous genotypes are associated with lower morphine CL in this extended cohort; well supported by corresponding observations of lower morphine glucuronide formation. In addition, it shows that children with the *ABCC3* –211C>T poly morphism C/C genotype have significantly higher (~40%) metabolite transformation than C/T+T/T genotypes.

With a growing focus on personalized medicine, there is an increased interest in quantifying the contribution of different PG factors to variability in opioid response [32-35]. Our primary focus of this study was to capture the role of PG of important transporters and enzymes on PK of intravenous morphine in children. In lieu of this aim, we developed a nonlinear mixed effects model to capture the time profiles for morphine and its metabolites by using approximately 500 plasma samples obtained from 220 children undergoing outpatient adenotonsillectomy. Using weight normalized estimates allowed us to study the effect of genetic polymorphisms in the absence of body size as a confounding factor. We have identified SNPs across three different transporters (*OCT1*, *ABCC3* and *ABCB1*) that contribute significantly to morphine and/or its metabolite PK in children, though no association with the *UGT2B7* and *ABCC2* genotype was found. Overall these results suggest that uptake and efflux transporters within hepatocytes impact morphine metabolism and disposition significantly and needs further investigation.

The results of this larger cohort study confirmed our results from an earlier subset of this study with an *OCT1* homozygous group that had approximately 17% lower CL than wild-type and heterozygous combined. Consistent with our results, Tzvetkov *et al.*, in a study using plasma samples up to 24 h post dose, have demonstrated that subjects with two or more functionally defective alleles had significantly lower morphine CL than others [11]. Our study offers further evidence that limited uptake of morphine into hepatocytes with functionally defective *OCT1* genotype directly impacted morphine CL. Consistent with the observation of lower CL, we, for the first time, also demonstrate that the *OCT1* homozygous subjects had lower transformation of both metabolites, M3G and M6G. Limited morphine uptake into hepatocytes in these subjects impacted downstream morphine's metabolic processes resulting in lower morphine glucuronide generation. Correlated observations of lower morphine CL as well as M3G/M6G formation among homozygous subjects provide stronger evidence that OCT1 uptake of morphine into hepatocytes plays a key role in the PK of morphine.

At physiologic pH, morphine is expected to be in an ionized state with the amide group charged ($pK_a = 8.4$ [36]), making it a suitable substrate of OCT1. *In vitro* studies have suggested that morphine has low transporter independent permeability and transporter dependent uptake of morphine into hepatocytes accounts for approximately 60% of the total uptake [11]. Furthermore, these studies also showed that morphine uptake was concentration dependent in hepatocytes overexpressing wild-type OCT1, although uptake rate was substantially reduced (75–100%) when loss-of-function polymorphisms were present. Based on our results, the impact of the presence of defective alleles was higher in metabolite formation (~39%) than morphine CL (~14%), and is more consistent with the 37% lower AUC observed in subjects with two or more defective alleles [11]. Although morphine being a substrate of OCT1 is well characterized, the role of OCT1 in the uptake of glucuronide metabolites is not known. This indicates that the effect of OCT1 on morphine CL might possibly be higher than the currently estimated 14% implying that homozygous subjects would experience better analgesia and possibly more adverse effects in response to just standard weight based morphine dosing in children. Further clinical data might be required to establish the contribution of the presence of OCT1 defective alleles in morphine PK and clinical responses.

In addition, our study showed that children with the C/C genotype of rs4793665 (*ABCC3*) had 46% higher M6G formation and 41% higher M3G formation indicating an increased efflux of metabolites into the plasma than C/T and T/T genotypes combined. *ABCC3* was found to be expressed primarily in the basolateral surface of the hepatocytes [37] and has been reported to have high affinities for morphine glucuronides [14]. Subjects with the TT genotype of the *ABCC3* –211C>T SNP have been reported to have decreased mRNA expression in the liver tissue compared with the CC genotype, possibly owing to the reduced binding of nuclear proteins to the promoter region of the gene [15,16]. Although the genotype was observed to clearly alter the metabolite formation, an increased morphine CL among subjects was also observed. To our knowledge no clear evidence of morphine being a substrate of *ABCC3* exists, although the efflux of morphine into the plasma by *ABCC3* has been speculated [11]. The observed effect of the –211C>T genotype on morphine CL could

be a second order effect observed upstream mainly due to a significant role of ABCC3 in the efflux of glucuronides into the plasma. Further studies including *in vitro* and *in vivo* studies along with physiologically based modeling efforts [38] are needed.

ABCB1, which in hepatocytes is expressed in the canalicular site is known to transport morphine and M6G, although evidence for M3G transport is sparse with few studies suggesting M3G not being a substrate in mice [8-10,13]. In our study, the presence of either allele in the synonymous *ABCB1* SNP, 3435C>T, did not have any discernable effect on morphine CL and M6G formation. However, subjects with the TT genotype had lower M3G formation than CT and CC genotypes combined. Altered biliary/renal excretion of M3G has been hypothesized to cause an approximately 10% increase in clinical M3G AUC and C_{max} (with unaltered morphine and M6G PK) [39] when ABCB1 was inhibited. Conversely, CSF accessibility in patients with the CC genotype was reported to be increased for morphine only with no effect on metabolites, although the study was smaller (n = 9) with only one subject having the CC genotype [22]. As the pharmacodynamic effects after morphine dosing are believed to be primarily due to morphine and M6G, the clinical impact of altered M3G PK due to *ABCB1* genotype is still uncertain and further investigations are needed.

Incidence of adverse effects after morphine dosing has been reported to be higher in Caucasian than African–American children and Latino than non-Latino children [1,2,40]. We have reported racial differences in morphine requirement observed in the clinic with African–American children requiring more morphine compared to Caucasian children [1]. We found that Caucasians had 8% lower morphine CL than African–Americans, which was further reduced to 7% when *OCT1* genotype was included as a covariate along with race. Also, our PK/PG associations were consistently reproduced when tested among majority race (Caucasians: 80%), thereby limiting the influence of population stratification (Supplementary Figure 7). As we reported earlier, the higher frequencies of the *OCT1* homozygous group among Caucasians (~5%) as compared to African–Americans [3] could be one of the reasons behind lower morphine CL in Caucasian children [2].

Frequency of the –900A>G genotype in the *UGT2B7* gene is known to be very different across races [41]. In our study, this common *UGT2B7* genotype was found to have little to no impact on morphine CL (Supplementary Figure 4). Further analysis is required to evaluate the effect that lower morphine CL and M3G/M6G FCL has on pharmacodynamics due to *OCT1* SNP frequency differences among different races.

Caucasian girls have more postoperative adverse effects with opioids such as respiratory depression and vomiting than boys with higher doses of morphine. In this study we observed a small reduction in morphine CL in girls compared to boys, which could partly explain this differential sex-related pharmacodynamics difference. A major limitation in our study was that the PK sampling was limited to 45 min after initial morphine dose owing to the short duration of this common outpatient surgery in children. Longer time plasma sampling is necessary for proper characterization of the elimination phase to make inferences on morphine and metabolite CLs. However, the morphine population mean CL estimated from this large cohort (n = 220) is similar to other studies [28,42]. Another limitation of our study is the lack of metabolite levels in all patients, lowering the statistical power of our results.

However, the pharmacogenetic effect of *OCT1* and *ABCC3* genotypes on morphine CL and metabolite transformations are consistent with current mechanistic understanding of morphine metabolic pathways. Nevertheless, further confirmation of these results using longer term sampling is needed.

Conclusion & future perspective

In conclusion, our large pediatric PK and PG study demonstrates that besides bodyweight, *OCT1* and *ABCC3* homozygous genotypes play a significant role in the PK of morphine and its metabolites. Specifically, children with the *ABCC3* rs4793665 homozygous C/C genotype had an approximately 46% higher M6G formation rate than the wild-type and heterozygous genotypes combined, resulting in increased M6G transport into the plasma. *OCT1* homozygous genotypes (n = 13) were found to have lower morphine CL (~14%) in our extended cohort, which is further supported by their having significantly lower metabolite formation. Higher frequencies of the *OCT1* homozygous genotypes were observed in the Caucasian population. This finding partially explains lower morphine CL and higher incidences of adverse effects with morphine in Caucasians than African-American children. Compared to boys, girls had lower morphine CL and this difference was small.

To personalize morphine use to an individual patient, PK variations due to multiple genes including *OCT1* and *ABCC3*, race and sex need to be considered. Variations in the PK of morphine and its metabolites need to be associated with clinically important outcomes in future studies to guide personalized dosing of morphine in children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Executive summary

Interindividual differences in morphine pharmacokinetics can be explained by *OCT1* & *ABCC3* genotypes

- In addition to genetic variants of *OCT1*, the *ABCC3* polymorphism –161C>T alters the pharmacokinetics (PK) of morphine and its metabolites in children undergoing adenotonsillectomy.

Role of *OCT1* genotypes & morphine PK

- *OCT1* is an uptake transporter that is known to play a significant role in uptake of morphine into hepatocytes. This study's extended cohort offers further supporting evidence that *OCT1* homozygous genotypes with functionally defective polymorphisms are associated with lower morphine clearance and demonstrates complementary lower morphine metabolite formation.
- In addition, *OCT1* homozygous genotype subjects had lower formation of morphine glucuronides in children.

Role of *ABCC3* genotype & morphine PK

- *ABCC3* is an efflux transporter expressed primarily on the basolateral surface of hepatocytes and has been known to efflux morphine glucuronides into the plasma.
- Children with the *ABCC3* –161C>T homozygous C/C genotype had higher morphine glucuronide formation than the wild-type and heterozygous genotypes.

Racial difference in morphine PK & clinical effects may be partly explained by *OCT1* & *ABCC3* genotypes

- *OCT1* alleles associated with decreased *OCT1* function are more common in Caucasians than in African-Americans, which could partly explain lower morphine clearance and relatively higher morphine related adverse effects in Caucasian children compared with African-Americans.

Potential clinical impact

- Incorporation of pharmacogenetic knowledge regarding transporters into the clinical setting may yield better personalization of intravenous morphine dose to optimize clinical response. Clinical studies with personalized intravenous morphine dosing need to consider genotypes of *OCT1* and *ABCC3* transporters in addition to bodyweight, age, sex, race and other important factors.

Figure 1. Hepatocyte uptake, metabolism, biliary efflux and efflux into plasma of morphine and its two prominent metabolites, morphine-3-glucuronide and morphine-6-glucuronide
Morphine is metabolized to M3G and M6G by UGT1A1 and UGT2B7 [43-45]. Morphine has been demonstrated to be an OCT1 substrate though the role of OCT1 in the uptake of M3G/M6G has not been reported [11]. ABCC3 is known to transport morphine glucuronides back into plasma while morphine has not been reported to be its substrate [14]. ABCB1, primarily expressed on the canalicular side is known to transport morphine and M6G, though M3G has been reported to not be a substrate [8-10,13]. ABCC2, expressed on the canalicular side, transports morphine glucuronides in mice into bile [7,12], while morphine is not known to be substrate.
M3G: Morphine-3-glucuronide; M6G: Morphine-6-glucuronide.

Figure 2. Pharmacokinetic model for morphine, morphine-3-glucuronide and morphine-6-glucuronide

Morphine pharmacokinetics were characterized using two compartments (parameterized using CL , V_1 , Q and V_2). The delay in the formation of metabolites was modeled using a hypothetical delay compartment. Mass transfer between the central compartment and the delay compartment was modeled using a single rate constant (k_{e0}). The metabolite concentrations were modeled using one compartment each, with metabolite formation and clearance modeled using FCL (FCL_{M3G} and FCL_{M6G}), V (V_{M3G} and V_{M6G}) and CL (CL_{M3G} and CL_{M6G}).

CL : Clearance; FCL: Formation clearance; M3G: Morphine-3-glucuronide; M6G: Morphine-6-glucuronide; Q : Intercompartmental clearance; V : Volume of distribution.

Figure 3. Plasma concentration–time profiles for morphine, morphine-3-glucuronide and morphine-6-glucuronide observed in the current study cohort

All plots are in the semi-log scale and concentrations are in ng/ml.

Conc: Concentration; M3G: Morphine-3-glucuronide; M6G: Morphine-6-glucuronide.

Figure 4. Variation of weight normalized morphine clearance and morphine-3-glucuronide and morphine-6-glucuronide formation clearances with *OCT1*, *ABCC3* and *ABCB1* genotype observed in our study

OCT1 genotype has been divided into three groups (defined in Table 1).

CL: Clearance; HM: Homozygous; HT: Heterozygous; M3G: Morphine-3-glucuronide;

M6G: Morphine-6-glucuronide; WT: Wild-type.

Table 1
Patient demographics across different races

Patient characteristics	All	Caucasian	African–American
Number of subjects (n)	220	176	38
Weight (kg) [†]	37.8 ± 14.5 (16.7–76.4)	37.5 ± 14.5 (16.7–76.4) [‡]	40 ± 15.2 (21.5–73.3) [‡]
Height (cm) [†]	137.4 ± 14.8 (97.8–181)	137.2 ± 15.1 (97.8–181) [‡]	138.3 ± 14.1 (116.2–176) [‡]
Age (years) [†]	9.2 ± 2.5 (6–15.9)	9.1 ± 2.5 (6–15.9) [‡]	9.2 ± 2.4 (6–14.8) [‡]
Gender (male/female) [§]	115 (52)/105 (48)	89 (51)/87 (49) [¶]	23 (61)/15 (39) [¶]
OSA status [#]	117 (53)	86 (49)	28 (74)
Comedication status [#]	126 (57)	98 (56)	26 (68)

OSA: Obstructive sleep apnea.

[†] Mean ± standard deviation (range).

[‡] No significant difference was observed between Caucasian and African–American populations based on t-tests.

[§] Number of boys (% boys)/number of girls (% girls).

[¶] No significant difference was observed in the gender ratio between Caucasian and African–American populations based on χ^2 test.

[#] Number (%).

Table 2
List of *OCT1* genotypes and their frequencies across different races observed in the current study

Group	Genotype	Nucleotide changes				All races			Caucasians			African-American		
		1365 GAT>del	1306 G>A	286 C>T	1498 G>C	n	n _{total}	Fraction	n	n _{total}	Fraction	n	n _{total}	Fraction
Wild-type	<i>OCT1</i> *1/*1	GAT	G	C	G	127	127	0.58	94	94	0.53	31	31	0.82
Het	<i>OCT1</i> *1/*2	GAT/del	G	c	G	43	80	0.37	37	70	0.40	4	7	0.18
	<i>OCT1</i> *1/*3	GAT	G	C/T	G	22			20			2		
	<i>OCT1</i> *1/*4	GAT	G/A	C	G	7			7			0		
	<i>OCT1</i> *1/*5	GAT/del	G	C	G/C	8			6			1		
Homo	<i>OCT1</i> *2/*2	del	G	C	G	2	13	0.06	2	12	0.07	0	0	0
	<i>OCT1</i> *2/*3	GAT/del	G	C/T	G	6			5			0		
	<i>OCT1</i> *2/*4	GAT/del	G/A	C	G	2			2			0		
	<i>OCT1</i> *2/*5	del	G	C	G/C	1			1			0		
	<i>OCT1</i> *3/*4	GAT	G/A	C/T	G	1			1			0		
	<i>OCT1</i> *4/*4	GAT	A	C	G	1			1			0		
Total						220			176			38		

Het: Heterozygous; Homo: Homozygous.

Table 3
Observed frequencies of *ABCC3* –211C>T (rs4793665) and *ABCB1* 3435C>T (rs1045642)
across different races in the current study

Gene	Group	Genotype	<u>All races</u>		<u>Caucasians</u>		<u>African-American</u>	
			n	%	n	%	n	%
<i>ABCC3</i> : –211C>T	Wild-type	T	70	0.32	58	0.33	11	0.29
	Het	C/T	112	0.51	90	0.51	19	0.5
	Homo	C	38	0.17	28	0.16	8	0.21
	Total		220		176		38	
<i>ABCB1</i> : 3435C>T	Wild-type	T	63	0.29	61	0.35	2	0.05
	Het	C/T	87	0.4	72	0.41	11	0.29
	Homo	C	70	0.32	43	0.24	25	0.66
	Total		220		179		38	

Het: Heterozygous; Homo: Homozygous.

Table 4
Model parameter estimates for the morphine and metabolite population pharmacokinetic model with allometric scaling

Parameter	NONMEM model		Nonparametric bootstrap	
	Estimate	95% CI [†]	Median	95% CI [‡]
Population mean parameters				
CL	1.3	1.19–1.40	1.29	1.18–1.40
V ₁	6.28	3.95–8.62	6.28	4.16–9.13
Q	2.1	1.58–2.618	2.09	1.52–2.61
V ₂	29.31	23.79–34.83	29.23	23.21–34.86
FCL _{M3G}	0.34	0.26–0.42	0.34	0.27–0.44
V _{M3G} [§]	23	–	–	–
CL _{M3G} [§]	0.29	–	–	–
FCL _{M6G}	0.045	0.034–0.056	0.044	0.035–0.059
V _{M6G} [§]	30	–	–	–
CL _{M6G} [§]	0.097	–	–	–
K _{delay}	0.069	0.041–0.097	0.069	0.045–0.10
Interindividual variability				
$\omega^2_{CL, M}$	0.052	0.029–0.076	0.052	0.029–0.079
$\omega^2_{FCL, M3G}$	0.3	0.22–0.38	0.30	0.22–0.37
$\omega_{FCL, M6G}$	0.36	0.23–0.48	0.35	0.23–0.47
Residual error				
ε_M	0.21	0.18–0.23	0.20	0.18–0.23
ε_{M3G}	0.38	0.33–0.42	0.38	0.33–0.42
ε_{M6G}	0.34	0.28–0.41	0.34	0.28–0.40

ω^2 : Between subject variance; ε : Proportional error coefficient; CL: Clearance; FCL: Formation clearance; M: Morphine; M3G: Morphine-3-glucuronide; M6G: Morphine-6-glucuronide; Q: Intercompartmental clearance; V: Volume.

[†] Confidence interval estimated based on standard error estimates.

[‡] The 2.5th and 97.5th percentile of the bootstrap parameter estimates.

[§] These parameters were fixed based on prior reports and were not estimated by NONMEM.

Table 5
Results of the pharmacogenetic covariate analysis that studied the impact of *OCT1*, *ABCC3* and *ABCB1* genotypes on morphine clearance and metabolite formation clearances

Transporter	Analysis method	Parameter	Morphine	Morphine-3-glucuronide	Morphine-6-glucuronide
OCT1 Wild-type+Het/ Homo	Anova	CL [†]	-0.101 (-0.208 to 0.005)	-0.403 (-0.720 to -0.008)	-0.213 (-0.839 to 0.413)
	NONMEM	CL [†]	-0.137 (-0.26 to -0.02)	-0.39 (-0.63 to -0.15)	-0.246 (-0.90 to 0.404)
		OFV [‡]	-3.5	-6.5	-0.5
ABCC3 CT+TT/CC	Anova	CL [†]	0.047 (-0.02 to 0.114)	0.304 (0.108 to 0.501)	0.283 (0.041 to 0.526)
	NONMEM	CL [†]	0.069 (-0.018 to 0.156)	0.463 (0.047 to 0.879)	0.418 (-0.044 to 0.879)
		OFV [‡]	-1.9	-9.5	-5.3
ABCB1 CC+CT/TT	Anova	CL [†]	-0.018 (-0.074 to 0.038)	0.178 (0.007 to 0.349)	0.101 (-0.119 to 0.32)
	NONMEM	CL [†]	-0.026 (-0.098 to 0.046)	0.248 (0.004 to 0.492)	0.135 (-0.153 to 0.422)
		OFV [‡]	-0.4	-4.3	-0.9

Results highlighted in bold were found to be statistically significant.

Cov: Model with genetic covariate included; Het: Heterozygous; Homo: Homozygous; NoCov: Model without the inclusion of genetic covariate; OFV: Objective function value.

[†] Mean (lower 95% CI to upper 95% CI).

[‡] OFV = OFVCov – OFVNoCov.