Research Article

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Pharmacogenomics

Novel associations between *CYP2B6* polymorphisms, perioperative methadone metabolism and clinical outcomes in children

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Aim: Methadone exhibits significant variability in clinical response. This study explores the genetic influence of variable methadone pharmacokinetics. **Methods:** This is a prospective study of methadone in children undergoing major surgery. *CYP2B6* genotyping, plasma methadone and metabolite levels were obtained. Clinical outcomes include pain scores and postoperative nausea and vomiting (PONV). **Results:** *CYP2B6* poor metabolizers (*6/*6) had >twofold lower methadone metabolism compared with normal/rapid metabolizers. The incidence of PONV was 4.7× greater with *CYP2B6 rs1038376* variant. *AG/GG* variants of *rs2279343* SNP had 2.86-fold higher incidence of PONV compared with the wild variant (*AA*). Nominal associations between *rs10500282, rs11882424*, *rs4803419* and pain scores were observed. **Conclusion:** We have described novel associations between *CYP2B6* genetic variants and perioperative methadone metabolism, and associations with pain scores and PONV.

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Keywords: analgesia • children • CYP2B6 • methadone • pharmacogenetics • pharmacokinetics • PONV

Methadone is a semisynthetic opioid agonist, with additional action on N-Methyl-D-aspartate (NMDA) pathway and also, serotonin-norepinephrine reuptake inhibitory (SNRI) property [1–3]. Methadone has been an important component of maintenance therapy for opioid use disorders (OUD). Along with its long duration of action, methadone is promising in terms of protection against chronic post-surgical pain, minimal abuse potential, minimal risk of opioid tolerance and opioid-induced hyperalgesia [4,5]. The analgesic potential of methadone is being increasingly recognized in the perioperative environment, both in adults and children, over the past two decades. The main barrier for widespread implementation has been the risk of life-threatening respiratory depression, QTc prolongation and deaths that have been associated with the use of methadone [6]. Additionally, methadone dosing strategy for perioperative use has largely been speculative, with most authors using a single large dose administered intra-operatively [5].

Methadone elimination is primarily hepatic, via the CYP enzymes. Though a number of CYP enzymes including CYP2B6, CYP2D6, CYP2C19, CYP2C9 and CYP3A4 have been described in methadone metabolism [7], CYP2B6 seems to be the most significant enzyme mediating stereoselective N-demethylation of both R- and S-methadone enantiomers, to their major and a number of minor metabolites, which are all inactive [8]. CYP2B6 expression shows 20–250-fold variability [9]. Variants in *CYP2B6* have been shown to significantly impact methadone PK in adults [10,11]. *CYP2B6 *6/*6* was associated with 45% lower clearance of S-methadone and 30% lower clearance of R-methadone compared with the wild-type, **1/*1*, in 64 healthy adults taking a single methadone dose [10].

Presence of *CYP2B6 *6/*6* alleles and subsequently high methadone concentrations have been demonstrated in cases of methadone-related fatalities [12].

Adult studies have associated *CYP2B6* polymorphism with methadone response and dose requirements in the setting of methadone maintenance therapy (MMT) for OUD [13,14], although this association is inconsistent across studies [15,16]. Understanding the role of *CYP2B6* genetic variants in the inter-individual variability in perioperative methadone's metabolism, analgesic and adverse clinical effects is essential to formulate personalized safe and effective surgical pain management and precision dosing. The current study aims to explore the influence of *CYP2B6* genetic variants on metabolism and clinical outcomes of perioperative methadone in children.

Materials & methods

Study setting

This is a prospective, genotype-blinded, clinical observational study of children undergoing Nuss bar repair for pectus excavatum (PE) and posterior spinal fusion (PSF) repair for idiopathic scoliosis, that received multidose methadone-based multimodal analgesia, performed in a tertiary care pediatric hospital. It aimed to study possible associations between *CYP2B6* genetic variants, methadone pharmacokinetics and clinical safety and efficacy outcomes of perioperative methadone.

Study approval

This study was approved by the Indiana University institutional review board (IRB #1707525204). Written informed consent was obtained from a parent or a legal guardian of each participant and written assent from the participant (>14 years of age). This study was registered at clinicaltrials.gov (NCT03495388; Date of registration: 28 March 2018) prior to enrollment.

Study participants

All patients aged 8–17.9 years of age undergoing PE and PSF surgeries were eligible for the study. Exclusion criteria included allergy to methadone, developmental delay, neurological disorder, liver and/or renal disease and preoperative pain requiring analgesics.

Analgesia protocol

Multimodal post-operative analgesia protocols specific to PE and PSF were standardized for all the participants undergoing the surgeries. Please refer to Supplementary Table 1 for detailed and standardized description of multimodal analgesia protocol that included multi-dose methadone regimen. Methadone was administered intraoperatively before the incision through the intravenous route (dose 1), and postoperatively through the oral route every 12 h for 3–5 doses at a dose of 0.1 mg/kg/dose up to a maximum of 5 mg (Supplementary Table 1).

Specimen collection, storage & analysis

Blood samples were collected at specific time windows after methadone administration (dose 1 was administered IV intraoperatively; subsequent doses were oral, given at 12-h intervals): baseline-before methadone administration, for genetic analysis; 10–30 min, 2–6 h, 10–12 h after dose 1 and 3; 1–2 h, 10–12 h after dose 2 through a designated intravenous (IV) line *in situ* for pharmacokinetic analysis, concentration-time profile of methadone and metabolites. R- and S-methadone and their corresponding metabolites R- and S-EDDP were measured in plasma using HPLC-MS/MS (Sciex 6500 + QTRAP) and a liquid-liquid extraction. The mass spectrometer utilized an electrospray ionization probe and was run in positive mode. The liquid-liquid extraction uses the addition of 0.1 M phosphate buffer (pH = 7.4) and ethyl acetate. The mobile phase is delivered via gradient using acetonitrile and 0.1% formic acid (pH = 6.5) on a Chiral Technologies Chiral-AGP 150 × 4.6 mm 5-micron column. The lower limit of quantification (LLOQ) was 0.15 ng/ml for the drug and the metabolite. CV were <15% for all the QC for all these analytes. The baseline sample before methadone administration was obtained after IV catheter placement in the operative room (OR), this was used for genotyping. DNA was isolated on the same day and frozen at -20°C.

Genotyping

Genome sequencing analysis was done at 40× coverage (mapped to hg 19) to provide adequate read depth to cover all the relevant genetic variants of *CYP2B6* and to accurately detect the copy numbers. Aldy1, a high-throughput sequencing data analysis tool with a combinatorial optimization framework to resolve allelic decomposition of highly

polymorphic *CYP2B6* gene was used to identify the genotypes from the mapped data. Sixteen previously studied common *CYP2B6* single nucleotide polymorphisms (SNP), with minor allele frequency ≥5% were selected to be included in the analysis. These included *rs4803419, rs2279344, rs1038376, rs10403955, rs10500282, rs10853744, rs11882424, rs2279342, rs2279343, rs2279345, rs3745274, rs707265, rs7250601, rs7250991, rs8100458* and *rs8192719*. These SNPs were assessed for linkage disequilibrium (LD) and those in strong LD were deemed redundant and excluded from the analyses. Eight SNPs were included in the final analyses (*rs4803419, rs2279344, rs1038376, rs10500282, rs11882424, rs2279342, rs2279343, rs8100458*).

Aldy1 output was used to classify genes coding for CYP enzymes as their corresponding phenotypes – rapid metabolizers (RM), normal metabolizers (NM), intermediate metabolizers (IM) and poor metabolizers (PM). The phenotypes were assigned according to the CPIC guidelines for Efavirenz (2009) [17] and the available resources on the activity of individual alleles on the PharmVar Webpage [18,19]. Accordingly, the **1, *2* and **5* are normal function alleles, **6, *9*, and **26* are known to be reduced function alleles, **18* is a no-function allele, **22* is an increased function allele. **10, *11* and **15* are alleles with uncertain function. These metabolizing phenotypes were analyzed for outcome associations as well.

Clinical outcome measures

The primary clinical outcome measure was postoperative nausea and vomiting (PONV). The secondary outcome measure was maximum postoperative pain scores. The number of episodes of PONV were recorded from nursing reports of nausea/vomiting and/or requirement of antiemetic intervention on electronic health records, for up to 48 h in the postoperative period or until patient discharge, whichever was earlier. Analgesic efficacy was described in terms of patient self-reported pain scores. Pain scores were collected every 2–4 h using a self-reported numeric rating scale ranging from 0 to 10; where '0' represents no pain and '10' represents the worst possible pain.

Statistical analysis

Analyses were performed using the dominant, additive or recessive models to explore for possible associations. Suppose 'A' is the referent allele and 'a' is the alternative allele. Associations were tested under dominant model (i.e., AA vs Aa/aa), additive model (i.e., AA vs Aa vs aa) and recessive model (i.e., AA/Aa vs aa). Linear regression was used to investigate SNP associations for pain score. Poisson regression was used to investigate SNP associations for PONV. Unadjusted as well as covariate-adjusted analyses, adjusted for weight, race, age, surgery type and sex were performed. For primary outcome, PONV, we computed the SNP's rate ratios (i.e., exponential of the slope parameter in the Poisson regression), p-values and 95% CIs. For postoperative pain score, we computed the SNPs' effect (i.e., the slope parameter in the linear regression model), p-values and 95% CIs.

Methods for metabolite/methadone area under the ROC curve (AUC) ratio

AUC was calculated for methadone and its metabolites using the linear trapezoidal method. Metabolite/methadone AUC ratio was calculated as the ratio of the AUC of the metabolite to the AUC of methadone. For a given time interval (T1 – T2), the AUC can be calculated as follows:

$$\textit{Area under the curve } (AUC) = \frac{1}{2} \times (C1 + C2) \times (T2 - T1)$$

where C1 is the concentration at time T1 and C2 is the concentration at time T2.

$$\text{Metabolite Methadone ratio} = \frac{\text{AUC of the metabolite}}{\text{AUC of methadone}} \times 100$$

We tested the association under dominant model (i.e., AA vs Aa/aa) with metabolite/methadone AUC ratio. We used linear regression to investigate both R- and S-methadone metabolite/methadone AUC ratio. First, we conducted unadjusted analyses (i.e., simple linear regression). Second, we conducted covariate-adjusted analyses (i.e., multiple linear regression), which adjusted for weight, race, age, surgery and sex. In both analyses, we computed the SNPs' slopes, p-values and 95% CIs of the slopes.

Since we used eight SNPs for our final analysis, we used a Bonferroni correction for multiple comparisons for the primary outcome measure, which yielded a significance threshold of p = 0.00625 (p = 0.05/8 SNPs). We

Table 1. Characteristics of participants.

Variable	Frequency n (%)	Frequency n (%)
Race	Caucasian = 46 (87%)	Other = 7 (13%)
Surgery type	Pectus repair = 24 (45%)	Spine fusion = 29 (55%)
Gender	Boys = 27 (51%)	Girls = 26 (49%)
Variable	**Mean**	**SD**
Weight (kg)	56.5	13.7
AGE (years)	14	1.6
Length of hospital stay (days)	2.7	0.8
Morphine equivalent (mg/kg) (pectus)	0.7	0.2
Morphine equivalent (mg/kg) (PSF)	0.6	0.2

PSF: Posterior spinal fusion; SD: Standard deviation.

Table 2. *CYP2B6* genetic associations with methadone-induced nausea and vomiting, adjusted for covariates including weight, race, age, surgery type and sex.

SNP	Referent allele (frequency)	Minor allele (frequency)	Referent group	Alternative group	Unadjusted analysis			Covariate adjusted analysis		
					Rate ratio	p-value	95% CI	Rate ratio	p-value	95% CI
Dominant model										
rs2279343	A (0.94)	G (0.06)	AA	AG/GG	2.68	0.025	1.14–6.35	2.50	0.092	0.86–7.22
Additive model										
rs2279343	A (0.94)	G (0.06)	AA	AG	2.68	0.025	1.14–6.35	2.50	0.092	0.86–7.22
				GG	NA	NA	NA	NA	NA	NA
rs1038376	A (0.71)	T (0.29)	AA/AT	AT	0.79	0.622	0.31–2.03	1.33	0.612	0.44–4.00
				TT	4.26	0.001	1.76–10.27	3.56	0.011	1.33–9.55
Recessive model										
rs1038376	A (0.71)	T (0.29)	AA/AT	TT	4.7	0.00015	2.11–10.46	3.21	0.01	1.32–7.79

NA: Not applicable.

also report associations reaching threshold of 0.05 as nominally associated for secondary clinical outcome and metabolite/methadone AUC ratio.

Results

Demographics

A total of 53 children were recruited. Mean age of the participants was 14 years, and majority of the participants were Caucasians (87%). About half of the participants were females. 45% of the participants underwent pectus repair and 55% underwent a PSF (Table 1).

Phenotype associations with PONV & pain scores

Among the 53 participants, 30 were NM, 17 were IM, 4 were PM and 2 were RM. We were not able to find any significant difference between the different metabolizing groups and clinical outcomes with just 2 RMs and 4 PMs (p-values >0.1 for RM vs PM, NM + RM vs IM + PM comparisons for PONV, pain scores).

CYP2B6 variants & clinical outcomes

We identified novel *CYP2B6* genetic associations between methadone-induced PONV and *CYP2B6* SNPs, *rs1038376* (p = 0.001), and *rs2279343* (p = 0.025) as well as maximal post-surgical pain score (*rs11882424*, p = 0.007).

Genetic associations with PONV

We were able to find significant association between *rs1038376* variant and the incidence of PONV (Table 2); the incidence of PONV was up to 4.7-times greater in those with the variant allele (*TT*) compared with the reference

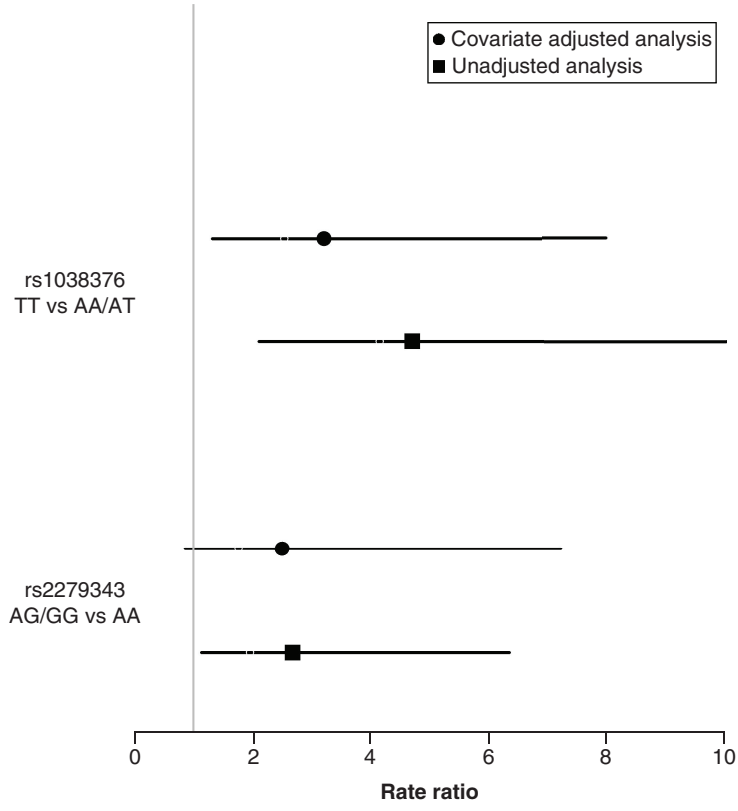

Figure 1. Rate ratios of PONV for variant versus reference alleles. The x-axis shows rate ratios of PONV, rate ratio >1 (marked by vertical line) means significantly increased risk of PONV. The *rs1038376* variant was associated with significantly greater risk of PONV, both in unadjusted (mean [95% CI]) 4.7 (2.11–10.46) and covariate adjusted models 3.21 (1.32–7.79). The *CYP2B6 rs2279343* and *rs1057868* variants showed greater risk of PONV, but the significance was nominal, on covariate adjustment.

Table 3. *CYP2B6* genetic associations with patient reported pain scores, adjusted for covariates including weight, race, age, surgery type and sex.

SNP	Referent allele (Frequency)	Minor allele (Frequency)	Referent group	Alternative group	Unadjusted analysis			Covariate adjusted analysis		
					Beta	p-value	95% CI	Beta	p-value	95% CI
Dominant model										
rs10500282	T (0.82)	C (0.18)	TT	TC/CC	1.16	0.020	0.19–2.13	1.18	0.024	0.16–2.20
rs11882424	T (0.95)	C (0.05)	TT	TC/CC	2.19	0.007	0.63–3.75	2.12	0.013	0.47–3.76
Additive model										
rs10500282	T (0.82)	C (0.18)	TT	TC	1.33	0.007	0.36–2.29	1.31	0.010	0.33–2.30
				CC	-2.00	0.234	-5.36–1.34	-2.61	0.156	-6.24–1.03
rs11882424	T (0.95)	C (0.05)	TT	TC	2.19	0.007	0.63–3.75	2.12	0.013	0.47–3.76
				CC	NA	NA	NA	NA	NA	NA
Recessive model										
rs4803419	C (0.65)	T (0.35)	CC/TT	TT	-1.78	0.03	-3.38– -0.19	-1.70	0.049	-3.39– -0.01

NA: Not applicable.

allele (*AA*/*AT*) (p = 0.00015) (Figure 1). On adjusting for covariates, this was reduced to nominal association (rate ratio: 3.21; 95% CI 1.32–7.79 [p = 0.01]). Similarly, *AG*/*GG* variants of *rs2279343* SNP exhibited up to 2.86-times greater episodes of PONV compared with the wild variant (*AA*) in the unadjusted models (Figure 1).

Genetic associations with pain scores

Nominal associations were detected between variants of *rs10500282, rs11882424* and *rs4803419* and maximum pain scores (Table 3). Those with *TT* variants of *rs4803419* reported lower pain scores compared with the wild type (*CC*). Those with variants of *rs10500282* and *rs11882424* reported greater pain scores compared with the referent group.

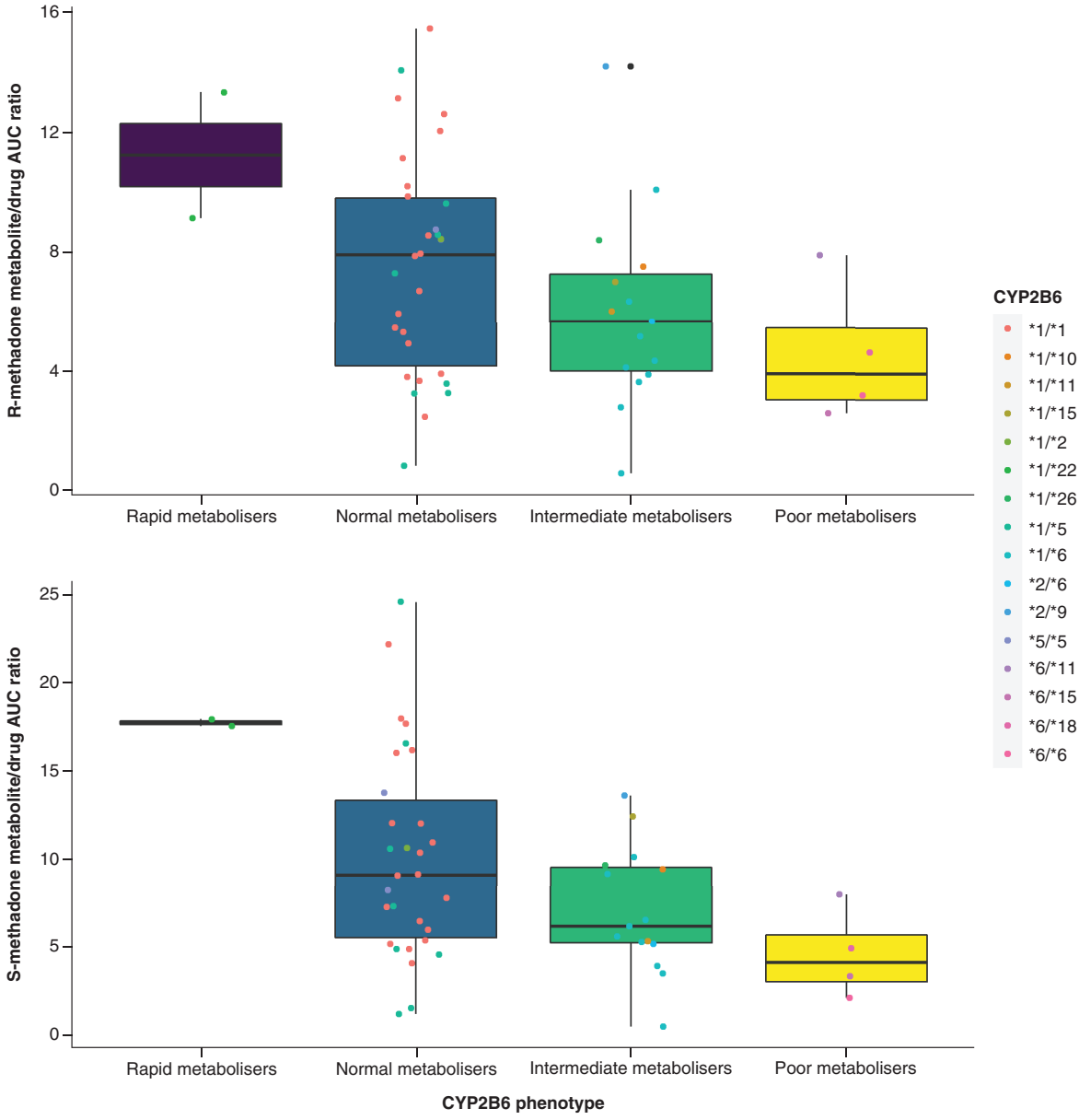

Figure 2. Phenotype-methadone pharmacokinetics association. Upper figure: CYP2B6 phenotype association with metabolite-drug ratio of R-methadone; Lower figure: CYP2B6 phenotype association with metabolite-drug ratio of S-methadone; Rapid metabolizers metabolize methadone over two-times faster than poor metabolizers, for both R and S enantiomers, the difference is more pronounced with the S-enantiomer.

CYP2B6 variants & methadone metabolizing status

CYP2B6 poor metabolizers (**6/*6*) had >twofold lower R- and S-methadone metabolites to methadone ratio compared with normal and rapid metabolizers (Figure 2). Nominal associations with metabolite/methadone AUC ratio were detected between variants of *rs10500282* (p = 0.012) and *rs1038376* (p = 0.032) in unadjusted analysis in additive models. In the co-variate adjusted model, the nominal significance persisted with *rs10500282* (p = 0.020) and no significant association noted with *rs1038376* (p = 0.079) (Table 4).

Discussion

This is the first study reporting novel associations between *CYP2B6* variants and methadone metabolism and clinical outcomes in a perioperative setting. Specifically, this manuscript shows that *CYP2B6* poor metabolizers (*6/*6) had >twofold lower R- and S-methadone metabolites to methadone ratio compared with normal and rapid

Table 4. *CYP2B6* genetic associations with methadone metabolite to methadone AUC ratio, adjusted for covariates including weight, race, age, surgery type and sex.

SNP	Referent allele (Frequency)	Minor allele (Frequency)	Referent group	Alternative group	Unadjusted analysis			Covariate adjusted analysis		
					Beta	p-value	95% CI	Beta	p-value	95% CI
Dominant model										
rs10500282	T (0.82)	C (0.18)	AA	AT/TT	-3.99	0.012	-7.05– -0.92	-3.78	0.020	-6.93– -0.63
rs1038376	A (0.71)	T (0.29)	TT	TC/CC	-3.29	0.032	-6.27– -0.30	-2.92	0.079	-6.18–0.35

metabolizers in adolescents who received multiple doses of methadone intraoperatively and postoperatively. More importantly, *CYP2B6 rs1038376* variant had significant association with high risk (4.7-fold) for PONV (Figure 1). In addition, *AG*/*GG* variants of *rs2279343* SNP had 2.86-fold higher incidence of PONV compared with the wild variant (*AA*). Maximum postoperative pain scores had nominal associations with *CYP2B6* variants, *rs10500282, rs11882424* and *rs4803419*. The allelic frequencies for these variants are tabulated in Supplementary Table 2, in comparison with population frequencies; we did not observe any significant deviation from Hardy–Weinberg equilibrium.

We have previously reported the safety and efficacy of the multiple small dose methadone regimen in a similar study cohort, compared with a historical cohort that received non-methadone-based analgesia [20]. We have also examined the pharmacokinetics of the same regimen and ascertained safe plasma methadone levels (<100 ng/ml) without any clinical respiratory depression even after repeated doses administered every 12 h. Other major concern that is specific to methadone is QT prolongation, which has been mostly reported in long term methadone maintenance therapy and use for chronic pain, where doses as high as 120 mg per day are used [21]. We did not observe any significant QTc prolongation in our cohort [22], given the small dose of methadone used, and the short duration of methadone therapy (2–3 days). The predominant adverse effect observed in this cohort was nausea, vomiting, that required antiemetic administration. In fact, about 40% of patients receiving opioids have been shown to experience nausea, and up to 25% may experience vomiting after opioid administration [23,24]. Patients rate nausea, vomiting after surgery worse than surgical pain [25]. Hence, we chose to use nausea, vomiting as the primary opioid adverse outcome, because of its patient-centric clinical relevance, as well as its relatively common incidence. Similarly, maximal pain scores were used as the measure of clinical effectiveness.

In our recent study, the peak methadone concentration (C_{max}) was 24.7 (IQR: 19.2–40.8) ng/ml and trough (C_{min}) was 4.09 (IQR: 2.74–6.4) ng/ml [22]. The wide range signifies significant inter-person variability in methadone metabolism. This could be the result of polymorphisms in the various enzymes involved in methadone metabolism and the fluctuating levels of the main binding protein: alpha-acid glycoprotein (AAG). Methadone undergoes hepatic metabolism, mainly via the CYP enzyme pathway. 10–20% of the parent drug is eliminated in urine [26]. It undergoes N-demethylation and spontaneous cyclization to form the major metabolites 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP), both of which have no opioid activity. The primary enzyme involved is the CYP2B6 [27]. Other enzymes involved in methadone metabolism include CYP3A4, CYP2D6, CYP2C9 and CYP2C219 [7,28]. Metabolism is stereoselective for R- and S-methadone [29]. CYP2B6 metabolizes S-methadone faster than R-methadone, while CYP2C19 metabolizes R-methadone preferentially than S-methadone [7].

Most of the pharmacogenetic studies on methadone have been performed in the context of methadone maintenance therapy (MMT) for opioid use disorders. Up to a 17-fold inter-individual variation in blood levels after methadone administration has been demonstrated, and the clearance varies between 0.02 and 2 l/min [30], and CYP2B6 has been a main contributing factor to this variable clearance. *CYP2B6* polymorphism has been associated to the variation in dose requirements for MMT [13,14]. But this finding has not been consistent across studies, due to the choice of end points used [15]. However, there is some agreement on the effect of *CYP2B6* on metabolism and clearance of methadone [8,31]. No previous studies have reported associations between *CYP2B6* variants and methadone's metabolism and clinical outcomes in perioperative settings, where AAG levels increase due to acute stress and influence methadone's immediate postoperative outcomes. In this study, we were able to explain methadone's metabolic and clinical outcome variations with *CYP2B6* genetic variants in children undergoing major inpatient surgeries.

CYP2B6 expression shows 20–250-fold inter-individual variability [9]. Genetic variants in *CYP2B6* have been shown to significantly impact methadone PK in healthy adults [32]. The most implicated allele is the **6* haplotype, which is a combination of **4* (*rs2279343*) and **9* (*rs3745274*) allelic variants. Kharasch *et al.*, in 64 healthy adult volunteers, have shown that *CYP2B6 *6/*6* was associated with 45% lower clearance of S-methadone and 30% lower clearance of R-methadone compared to the wild-type **1/*1* [32]. The authors used a single dose of methadone in this study. There was a 2.1-fold and 1.7-fold increase in trough and peak concentrations, respectively, of S-methadone in homozygous **6* carriers compared with the non-carriers and a 1.3-fold increase in both trough and peak plasma concentrations of R-methadone [11,33]. In our study that used multiple doses of methadone in children perioperatively, we show >200% lower methadone metabolite to methadone AUC ratio. Specifically, we compared CYP2B6 phenotypes for methadone metabolism (drug/metabolite ratios) and showed poor metabolizers (**6/*6*) had >twofold lower metabolite/drug AUC ratios, compared with normal (**1/*1*) or rapid metabolizers (**1/*22*) (Figure 2). But we did not observe these metabolic differences contributing to variations in clinical outcomes, which may be due a small number of extreme phenotypes (2 PM and 4 RM) in this study to detect any significant difference in clinical outcomes between phenotypes. This closely reflects the meta-analysis of seven articles, that found significant association between the **6* variant and decreased metabolism but failed to find any significant association with methadone dose requirements or response [31].

Since CYP2B6 effect on methadone metabolism is stereoselective, influencing (S) methadone greater than (R) enantiomer [10,34], the effect of *CYP2B6* polymorphism on the clinical response of methadone is unclear, as most if not all of the opioid receptor activity of methadone is attributed to the R enantiomer [30,35]. Further, other factors like varying levels of AAG, variants on other *CYP* genes, *POR* gene could affect PK of methadone [15,36,37]. It is possible that PD variants like *ABCB1*, *OPRM1* might contribute to variable responses to methadone as well [13,38–40].

CYP2B6 variant *rs1038376* was significantly associated with greater incidence of PONV after adjusting for multiple comparisons. *rs1038376* is a noncoding variant allele, that has been associated with increased concentrations of S-methadone, as part of a haplotype [41,42]. We also found *rs2279343* to be nominally associated with greater incidence of PONV. *CYP2B6*4 rs2279343* is a variant in the coding region of the *CYP2B6* gene [11,33,43], that has been associated with significantly greater methadone metabolism [32]. This apparent discrepancy might be because, greater metabolism and clearance of methadone in *rs2279343* variant, might impair analgesic efficacy of methadone, leading to greater use of PRN opioid medications. This was in fact, demonstrated in our previous study, where we found no association between PONV incidence and methadone levels, but a positive correlation between morphine equivalent dose of rescue opioids and the number of PONV episodes [22].

CYP2B6 polymorphisms have also been found to influence methadone dose requirements for MMT [13,14]. Homozygosity for variants *rs2279343* (*785A > G*) and *rs3745274* (*516G > T*) were found to be associated with significantly decreased methadone dosage requirements [14]. Though we did not find any significant association with *rs3745274* variant, *rs2279343* was associated with increased risk of PONV. This might probably be due to the basic difference in the population groups and the differences in the dose of methadone used. Long term methadone management in context of MMT has the potential for autoinduction of CYP2B6, which may not be relevant in postoperative setting. Further, the mean daily dose of methadone in this study was 140 mg per day, which is significantly higher than perioperative dosing [14], especially in our pediatric study. Further, genetic ancestry seems to play a major role in pharmacogenetic associations, as the above study predominantly consisted Middle Eastern/European ancestry, compared with primarily Caucasian subjects in our study. This factor is further highlighted by another study in Han Chinese population, where *rs3745274* variant was associated with increased methadone dose requirements [13].

In the context of analgesic efficacy, when used postoperatively, we were able to find nominal associations of *rs10500282, rs11882424* and *rs4803419* with patient reported maximum postoperative pain scores. All the above three are noncoding variants [41,42,44]. *rs11882424* affects the clearance of methadone, but the directionality of this association is unclear [41]. *rs10500282* has been associated with an increased S-methadone concentration as part of a haplotype [42]. The significance of this nominal association is currently unclear.

The current study is an exploratory study, looking for *CYP2B6* variants that might contribute to perioperative methadone's metabolic variations, and variations in analgesic efficacy and adverse events. The study's small sample size of 53 children had adequate statistical power to compare only the primary outcome measure, PONV with *CYP2B6* variants. Our study did not have adequate power to compare secondary outcomes and metabolic phenotypes, due to a small number of extreme phenotypes (RM and PM). We were able to identify some novel

genetic associations of nominal significance, that will provide preliminary data and effect size for power calculation, for future adequately powered hypothesis driven studies. Clinical correlation of phenotypes to outcomes might be a more viable approach from clinical perspective than SNP association, and this would require much larger sample size. Further, CYP2B6 in only one of the many enzymes that mediate methadone metabolism, others being CYP3A4, 2C19, 2D6, 2C18, 3A7, 2C8, 2C9 and 3A5 [28]. Given the stereoselective metabolism of methadone, other genetic variants may have the potential to impact methadone's pharmacokinetics. CYP enzymes (3A4, 3A5 and 3A7) require electron transfer through the P450 oxidoreductase (*POR*) [37]. Polymorphisms in POR is also being investigated toward inter-person variability in methadone response. Polymorphisms in PD genes, such as *OPRM1* and *ABCB1* can have profound impact on clinical response to opioids as shown in our previous studies with morphine [45,46]. The current study is a first step toward the final goal of a multi-gene model to predict methadone response in children to enable tailored administration.

In summary, we have described novel associations between *CYP2B6* polymorphisms, variations in methadone's metabolism and clinical outcomes of perioperative doses of methadone. *CYP2B6 rs1038376* and *rs2279343* were associated with greater risk of methadone induced PONV. *CYP2B6* variants of *rs10500282*, *rs11882424* and *rs4803419* showed association with maximum pain scores. *CYP2B6* variants, *rs10500282* and *rs1038376* were associated with metabolite/methadone AUC ratio. This study attempts to provide a glimpse into the pharmacogenetics affecting perioperative methadone. There is an expanding knowledge-base for feasibility of genotype guided personalized medicine [47–49]. In the context of methadone, this means, understanding the contribution of other CYP450 enzymes involved in the metabolism of methadone and the role played by polymorphisms of genes such as *OPRM1, ABCB1* etc. that influence the pharmacodynamics of methadone. This would be a potential direction for future research.

Supplementary data

To view the supplementary data that accompany this pape please visit the journal website at: www.futuremedicine.com /doi/suppl/10.2217/pgs-2021-0039

Author contributions

All authors contributed to the drafting of the work or revising it critically for content. All authors have reviewed and approve of this manuscript for submission. All authors agree to be accountable for all aspects of the work.

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Summary points

- Methadone is a long-acting opioid agonist, which exhibits significant variability in metabolism, analgesic response and adverse effects.
- Pharmacokinetic and pharmacodynamic factors play a role in this variability, *CYP2B6* polymorphisms play a chief role in affecting variability in methadone metabolism.
- This is a prospective study of the influence of CYP2B6 polymorphism on metabolism, analgesia and postoperative nausea and vomiting (PONV) related to perioperative methadone-based regimen in adolescents undergoing posterior spine fusion and pectus repair.
- We were able to find novel associations between *CYP2B6* variants and perioperative methadone's metabolism and clinical outcomes.
- Metabolic phenotype analysis showed >twofold variation in methadone metabolism between *CYP2B6* rapid metabolizers and poor metabolizer groups, this did not translate to significant clinical differences between groups due to the small sample sizes of the extreme phenotypes.
- Incidence of PONV was 4.7-times greater in those with *CYP2B6 rs1038376* variant.
- *AG*/*GG* variants of *rs2279343 SNP* exhibited up to 2.86-times greater incidence of PONV compared to the wild variant (*AA*).
- Nominal associations were detected between variants of *rs10500282, rs11882424* and *rs4803419* and maximum postoperative pain scores.
- *CYP2B6* variants, *rs10500282* and *rs1038376* were associated with variations in metabolite/methadone AUC ratio.

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