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Methadone pharmacogenetics: CYP2B6 polymorphisms determine plasma concentrations, clearance and metabolism

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Abstract

Background—Interindividual variability in methadone disposition remains unexplained, and methadone accidental overdose in pain therapy is a significant public health problem. Cytochrome P4502B6 (CYP2B6) is the principle determinant of clinical methadone elimination. The *CYP2B6* gene is highly polymorphic, with several variant alleles. CYP2B6.6, the protein encoded by the *CYP2B6**6 polymorphism, deficiently catalyzes methadone metabolism *in vitro*. This investigation determined the influence of *CYP2B6**6, and other allelic variants encountered, on methadone concentrations, clearance, and metabolism.

Methods—Healthy volunteers in genotype cohorts *CYP2B6**1/*1 (n=21), *CYP2B6**1/*6 (n=20), and *CYP2B6**6/*6 (n=17), and also *CYP2B6**1/*4 (n=1), *CYP2B6**4/*6 (n=3), *CYP2B6**5/*5 (n=2) subjects received single doses of intravenous and oral methadone. Plasma and urine methadone and metabolite concentrations were determined by tandem mass spectrometry.

Results—Average S-methadone apparent oral clearance was 35 and 45% lower in *CYP2B6**1/*6 and *CYP2B6**6/*6 genotypes, respectively, compared with *CYP2B6**1/*1, and R-methadone apparent oral clearance was 25 and 30% lower. R- and S-methadone apparent oral clearance was 3- and 4-fold greater in *CYP2B6**4 carriers. Intravenous and oral R- and S-methadone metabolism was significantly lower in *CYP2B6**6 carriers compared with *CYP2B6**1 homozygotes, and greater in *CYP2B6**4 carriers. Methadone metabolism and clearance were lower in African-Americans due to the *CYP2B6**6 genetic polymorphism.

Conclusions—*CYP2B6* polymorphisms influence methadone plasma concentrations, due to altered methadone metabolism and thus clearance. Genetic influence is greater for oral than intravenous, and S- than R-methadone. *CYP2B6* pharmacogenetics explains, in part, interindividual variability in methadone elimination. *CYP2B6* genetic effects on methadone

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metabolism and clearance may identify subjects at risk for methadone toxicity and drug interactions.

Keywords

methadone; cytochrome P450 2B6; CYP2B6

Introduction

Methadone is a long-duration opioid for acute, chronic, perioperative, neuropathic, and cancer pain,¹⁻³ a cornerstone therapy for opioid addiction, and a public health strategy for HIV/AIDS and hepatitis C reduction.⁴ Methadone is typically a racemic mixture. R-methadone primarily confers the mu opioid receptor activity, while both enantiomers act at N-methyl-D-aspartate receptors.⁵ Clinical utility includes effectiveness in opioid-tolerance, pain, neonates-adults, with rapid onset, administration by multiple routes, high oral bioavailability, and no active metabolites. In the US, >300,000 patients in opioid treatment programs receive methadone annually.⁶ Methadone use for pain has grown markedly. Prescriptions increased 5-fold 2000-2009,⁷ with >5 million annually for pain,⁷ the majority of which are written by primary care and non-pain physicians.⁸

Opioid fatalities are a growing public health problem. Specifically, with expanded use, methadone fatalities increased > 5-fold, with methadone involved in approximately one-third of opioid-related overdose deaths 1999-2009.^{7,9} In 2009, methadone accounted for only 2% of prescriptions, but 30% of prescription painkiller deaths.⁷ Increased methadone mortality is attributed exclusively to pain use, and many deaths occur during induction of therapy.¹⁰ While drug interactions can influence methadone disposition,¹¹ mechanism(s) of constitutive (not influenced by drug interactions) variability in plasma concentrations remain unidentified, despite decades of inquiry. This considerable inter- and intra-individual variability in disposition is a vexing clinical problem which confounds reliable dosing, and can cause inadequate analgesia, withdrawal, or toxicity.¹² Understanding methadone disposition is important for reducing adverse events.

Methadone is cleared principally by hepatic cytochrome P450 (CYP)-catalyzed N-demethylation to inactive 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP), and some urinary excretion of unchanged drug. CYP2B6 is the principle determinant of clinical methadone elimination.^{3,13-16} This recent recognition has necessitated a paradigm shift in therapeutic use, and motivates renewed quests for understanding variability in methadone disposition.

The *CYP2B6* gene is highly polymorphic,¹⁷ with numerous single nucleotide polymorphisms responsible for thirty-eight variant alleles identified.¹⁸ The most common and clinically significant variant allele is *CYP2B6**6 (516G>T, Q172H; 785A>G, K262R), which encodes CYP2B6.6 protein, having markedly reduced hepatic expression and activity.¹⁷ *CYP2B6**6 is significant because it occurs commonly (particularly in Africans, Asians, and Hispanics) and influences important CYP2B6 substrates (e.g. efavirenz, bupropion, cyclophosphamide).^{17,19} Another notable, albeit less common variant is *CYP2B6**4 (causing increased expression and variably increased or decreased activity).¹⁷

Associations between one *CYP2B6* polymorphism and methadone disposition have been identified.²⁰ Specifically, a single dose-adjusted plasma S-methadone concentration at steady-state was greater in *CYP2B6**6 homozygotes than heterozygotes and non-carriers,²¹⁻²⁴ and methadone dose requirements were lower.²⁴⁻²⁶ Nevertheless, the mechanism (altered systemic clearance, hepatic clearance, hepatic metabolism, renal clearance, other) for these associations is unknown. We recently found that expressed *CYP2B6*.6 catalyzed substantially less methadone N-demethylation than wild-type *CYP2B6*.1.²⁷ Furthermore, liver microsomes from humans carrying the *CYP2B6**6 allele had diminished *CYP2B6* content and methadone N-demethylation.²⁷ Such *in vitro* findings suggested that *CYP2B6* variants might influence methadone elimination *in vivo*. Nevertheless, the influence of *CYP2B6**6, or other *CYP2B6* variants, on methadone metabolism and clearance, is unknown.

This investigation determined the influence of *CYP2B6* genetic variation, specifically *CYP2B6**6 polymorphism, on clinical methadone plasma concentrations, clearance, and metabolism. The hypothesis was that *CYP2B6**6 hetero- or homo-zygotes would have reduced metabolism and clearance. A secondary objective was to evaluate other, less common genotypic variants, when encountered. Healthy volunteers were genotyped, and then *CYP2B6* genotype cohorts composed to evaluate methadone disposition.

Materials and Methods

Research Subjects and Clinical Protocol

The investigation was approved by the Washington University in St. Louis Institutional Review Board. All subjects provided written informed consent. Inclusion criteria were age 18-50 yr normal healthy volunteers (smokers or nonsmokers) in good general health without remarkable medical conditions, within 30% of ideal body weight (body mass index <33). Exclusion criteria were a history of hepatic or renal disease, use of prescription or non-prescription medications, herbals or foods known to be metabolized by or affect activity of *CYP2B6*, known history of drug or alcohol addiction, routine handling of addicting drugs in the regular course of employment, and pregnant or nursing females.

Potential subjects provided a venous blood sample and genomic DNA was isolated from peripheral blood leukocytes using the Gentra Puregene blood kit (Qiagen, Germantown, MD). All were genotyped for the *CYP2B6* 516G>T (rs3745274), 785A>G (rs2279343), 983T>C (rs28399499), and 1459C>T (rs3211371) single nucleotide polymorphism (SNP). Genotyping was performed by the Genome Technology Access Center at Washington University in St. Louis, using the Fluidigm BioMark System. Primer sequences were: 516G>T (rs3745274) forward: CTTGACCTGCTGCTTCTTCCTA, reverse: AGACGATGGAGCAGATGATGTTG; 785A>G (rs2279343) forward: TGGAGAAGCACCGTGAAACC, reverse: TGGAGCAGGTAGGTGTTCGAT; 983T>C (rs28399499) forward: TGGTCTTCTTTTCTGTACAGAGAGAGT, reverse: GCGATGTGGGCCAATCAC; 1459C>T (rs3211371) forward: GTGTGGTGTGGGCAAAATACC, reverse: CTTCCCTCAGCCCCTTCAG. The 48×48 genotyping chip was primed using the IFC (Integrated Fluidic Circuit) Controller MX. Samples were loaded into the sample inlets of the chips mixed with universal PCR master

mix, 20× GT loading reagent, and AmpliTaq Gold Polymerase at 100ng/ul. The 40× Taqman genotyping assays were loaded with 2× assay loading reagent and ROX in the assay inlets, 6 replicates per assay. The samples and assays were loaded in the chips using the IFC MX. The chip was cycled using the Fluidigm BioMark. Results were loaded into Fluidigm SNP Genotyping Analysis Software for further analysis. Analysis of these SNPs permitted detection of the *CYP2B6* *1, *4 (785A>G), *5 (1459C>T), *6 (516G>T, 785A>G), *7 (516G>T, 785A>G, 1459C>T), *9 (516G>T), *16 (785A>G, 983T>C), and *18 (983T>C) alleles.

Genotyping results then were used to invite subject participation and create target cohorts of twenty subjects each with *CYP2B6**1/*1, *CYP2B6**1/*6 and *CYP2B6**6/*6 genotypes. A 30% difference between groups was considered clinically significant. To detect a 30% difference between *CYP2B6* genotypes, with 30% variability, $\beta=0.8$, and $\alpha=0.05$, would require 17 subjects per group. The target was 20 per group. In addition, subjects of other rare genotypes coincidentally identified were also studied. A total of 64 subjects (34 male, 30 female; 42 Caucasians, 10 African-Americans, 10 Asians, 2 other/unknown), 29 ± 8 yrs, 74 ± 13 kg, were studied. Detailed demographic data are provided in Table 1.

Subjects were instructed to refrain from: 1) alcohol for 48 hr prior to and during the study day, 2) caffeine-containing beverages on the study day, 3) oranges, grapefruit or apples or their juices for 5d before and throughout the 96 hr study period, 4) food/liquids after midnight the day prior to methadone administration, 5) non-study medications (including over the counter and/or herbal), for 3d prior to the study day, without prior approval.

Study design was a single-center, open-label, single-session protocol. Methadone disposition was assessed by simultaneously administering intravenous (IV) and oral methadone.^{13,15,28-32} Subjects had a peripheral IV catheter inserted in each arm, for blood sampling and IV drug administration. Subjects received IV ondansetron for antiemetic prophylaxis, followed 30 min later by IV racemic unlabeled (d0)-methadone HCl (6.0 mg, equivalent to 5.4 mg free base) and oral deuterated racemic (d5)-methadone HCl (11.0 mg, equivalent to 9.86 mg free base)²⁸ dissolved in water immediately before use, followed by 100 ml water. Subjects received a standard breakfast and lunch 2 and 4 hr after methadone, respectively, and free access to food and water thereafter. Venous blood was sampled for 96 hr after methadone, centrifuged, and plasma stored at -80°C. Continuous urine samples were collected at 24, 48, 72, and 96 hr and stored at -80°C. Nausea and/or vomiting were treated with ondansetron (4 mg IV or 8 mg orally) as needed. Subjects were monitored using pulse oximetry and noninvasive blood pressure cuff, as standard safety measures.

Plasma and urine methadone and EDDP enantiomer concentrations were quantified by chiral liquid chromatography-tandem electrospray mass spectrometry as described previously.¹⁵ Interday coefficients of variation for methadone and EDDP were 6-13% in plasma and 3-10% in urine.

Data and statistical analysis

Pharmacokinetic data were analyzed using noncompartmental methods (Phoenix, Pharsight Corp, Mountain View, CA), assuming complete absorption, as described previously.^{13,28-32}

Results are reported as the arithmetic mean $\pm$ standard deviation (SD). The primary outcome measure was methadone metabolism, measured as plasma EDDP/methadone area under the concentration-time curve (AUC_{0-96}) ratio and EDDP formation clearance. Secondary outcomes included methadone peak plasma concentration, exposure (plasma AUC_{∞}), methadone systemic, apparent oral, and hepatic clearance, and oral methadone bioavailability. For the main objectives, differences between *CYP2B6**1/*1, *CYP2B6**1/*6 and *CYP2B6**6/*6 genotypes for pharmacokinetic parameters were analyzed using one-way analysis of variance followed by the Student-Newman-Keuls test for multiple comparisons (Sigmaplot 12.5, Systat Software, Inc, San Jose, CA). Non-normal data were log transformed for analysis, but reported as the non-transformed results. Racial groups were compared using Student's t-test. Statistical significance was assigned at $p < 0.05$. Formal comparison of other *CYP2B6* allelic variants to *CYP2B6**1/*1 subjects was not performed due to the small subject numbers studied. Relationships between methadone clearance and metabolism were evaluated using the Pearson product moment correlation.

Results

Allele frequencies in the 489 subjects genotyped (*CYP2B6**4 0.02, *CYP2B6**5 0.07, *CYP2B6**6 0.22, *CYP2B6**7 0.02, *CYP2B6**9 0.002, *CYP2B6**16 0.002, *CYP2B6**18 0.016) are consistent with previous reports.^{17,19} Full cohorts of *CYP2B6**1/*1 and *CYP2B6**1/*6 genotypes were evaluated, although only 17 *CYP2B6**6/*6 subjects could be identified and studied. Other subjects identified with rare allelic variants were also evaluated, including one *CYP2B6**1/*4 heterozygote, three *CYP2B6**4/*6 heterozygotes, and two *CYP2B6**5/*5 homozygotes.

Plasma methadone and EDDP enantiomer concentrations are shown for oral (Figure 1) and intravenous (Figure 2) methadone, for the three major genotype groups (*CYP2B6**1/*1, *CYP2B6**1/*6, *CYP2B6**6/*6), and for *4 carriers (*CYP2B6**1/*4 and *CYP2B6**4/*6, shown together as *CYP2B6**4/X). Methadone concentrations were higher in *6 carriers, with a gene dose effect, and much lower in *4 carriers. Genotype influence was greater for oral than intravenous dosing, and for S- than R-methadone. For oral methadone, average plasma exposure (area under the curve, AUC_{∞} , ng/ml-hr) in *CYP2B6**1/*1, *CYP2B6**1/*6, and *CYP2B6**6/*6 cohorts was 620 ± 230 , 734 ± 245 , and 1242 ± 801 (*CYP2B6**1/*6 and *CYP2B6**6/*6 $p < 0.05$ vs *CYP2B6**1/*1) for S-methadone, and 578 ± 205 , 615 ± 172 , and 898 ± 507 (*CYP2B6**6/*6 $p < 0.05$ vs *CYP2B6**1/*1) for R-methadone, respectively. AUC_{∞} for *CYP2B6**4/X subjects was 155 ± 45 and 177 ± 48 for S- and R-methadone, respectively. *CYP2B6* genotype did not affect peak methadone concentrations. For intravenous methadone, AUC_{∞} in *CYP2B6**1/*1, *CYP2B6**1/*6, and *CYP2B6**6/*6 genotypes was 447 ± 85 , 513 ± 171 and 801 ± 464 (*CYP2B6**6/*6 $p < 0.05$ vs *CYP2B6**1/*1) for S-methadone, and 430 ± 131 , 429 ± 135 , and 570 ± 281 for R-methadone, respectively. AUC_{∞} for *CYP2B6**4/X subjects was 280 ± 125 and 296 ± 110 for S- and R-methadone, respectively.

Intravenous and oral methadone plasma concentrations in carriers of minor *CYP2B6* allelic variants is shown in Figure 3. *CYP2B6**5 homozygote concentrations resembled those of *CYP2B6**1 homozygotes, while *CYP2B6**1/*4 and *CYP2B6**4/*6 subjects had lower concentrations, particularly with oral methadone.

Methadone disposition was stereoselective, with greater initial exposure to S-methadone, and a time-dependent increase in the plasma methadone R/S concentration ratio (Figures 1 and 2). This ratio change was diminished in *CYP2B6**6 allele carriers, and accentuated in *CYP2B6**4 carriers.

Genotypic differences in methadone plasma concentrations were accounted for by differences in clearance. For intravenous drug, S-methadone systemic clearance (ml/kg/min) in *CYP2B6**1/*6 and *CYP2B6**6/*6 subjects (1.2 ± 0.4 and 0.96 ± 0.33 , respectively) was significantly less than in *CYP2B6**1 homozygotes (1.5 ± 0.3) (Figure 4). R-methadone clearances in *CYP2B6**6 carriers were not significantly different from *CYP2B6**1/*1 subjects. Hepatic clearance (ml/kg/min) was significantly less in *CYP2B6**6/*6 compared with *CYP2B6**1/*1 subjects for S-methadone (0.8 ± 0.4 and 1.3 ± 0.3) but not R-methadone (1.0 ± 0.3 and 1.3 ± 0.3), and this was also found for hepatic extraction ratios (S-methadone extraction 0.05 ± 0.02 and 0.08 ± 0.02 in *CYP2B6**6/*6 and *CYP2B6**1/*1 subjects) (not shown). For oral dosing, S-methadone apparent clearance in *CYP2B6**1/*6 and *CYP2B6**6/*6 subjects (1.6 ± 0.5 and 1.2 ± 0.6 , respectively) was significantly less than in *CYP2B6**1 homozygotes (2.3 ± 1.5) (Figure 4). R-methadone apparent oral clearance was also significantly less in *CYP2B6**6 than *CYP2B6**1 homozygotes 1.6 ± 0.7 vs 2.4 ± 1.2 , respectively). In contrast, in *CYP2B6**4/X subjects, R- and S-methadone systemic clearances (2.4 ± 0.7 and 2.7 ± 0.9) and apparent oral clearances (7.4 ± 3.8 and 8.6 ± 3.2) were numerically greater than *CYP2B6**1/*1 subjects. Oral bioavailability in *CYP2B6**1/*1, *CYP2B6**1/*6 and *CYP2B6**6/*6 subjects was not significantly different for S-methadone (75 ± 21 , 79 ± 14 , and $83 \pm 18\%$, respectively) or R-methadone (75 ± 20 , 80 ± 13 , and $84 \pm 16\%$), but was numerically lower in *CYP2B6**4/*X subjects (39 ± 21 and $34 \pm 19\%$, for R- and S-methadone) (not shown).

Genotypic differences in methadone clearance were accounted for by differences in metabolism. There was a significant correlation between methadone apparent oral clearance and N-demethylation (plasma EDDP/methadone AUC ratio; Pearson product moment correlation $r=0.57$ and 0.82 for R- and S-methadone, respectively, both $p<0.001$; not shown). Methadone N-demethylation, evaluated from both the plasma EDDP/methadone AUC ratio and EDDP formation clearance, for both intravenous and oral methadone, and for both enantiomers, was significantly lower in *CYP2B6**6 carriers compared with *CYP2B6**1 homozygotes (Figure 5). Conversely, N-demethylation was numerically greater in *CYP2B6**4/X than *CYP2B6**1/*1 subjects. For example, for intravenous methadone, in *CYP2B6**4/X and *CYP2B6**1/*1 subjects, R-EDDP formation clearances (ml/kg/min) were 0.45 ± 0.17 and 0.26 ± 0.10 , respectively, and S-EDDP formation clearances were 0.88 ± 0.36 and 0.43 ± 0.16 . Results were comparable for oral methadone. Intravenous methadone renal clearance was not affected by *CYP2B6* genotype (not shown).

Additional analysis of oral methadone pharmacokinetics was based on race, comparing Caucasians and African-Americans (Table 2). *CYP2B6* allele frequencies in the two groups are similar to those reported previously.¹⁷ For both R- and S-methadone, apparent oral clearance and N-demethylation (both plasma EDDP/methadone AUC ratio and EDDP formation clearance) were significantly lower in African-Americans. This appeared related to the proportionally greater number of *CYP2B6**6 carriers and/or the absence of *CYP2B6**4

carriers in the African-Americans. When *CYP2B6**4 carriers were omitted from the analysis, R- and S-EDDP formation clearance was still significantly lower, and R- but not S-methadone apparent oral clearance was lower, in the African-Americans. Thus both *CYP2B6**4 and *CYP2B6**6 may contribute to differences in methadone elimination between Caucasians and African-Americans.

Discussion

The major finding of this investigation is that *CYP2B6* genotype affects methadone plasma concentrations, clearance, and metabolism. *CYP2B6**6 allele carriers, particularly homozygotes, had higher methadone concentrations and slower elimination, while *CYP2B6**4 carriers had lower concentrations and faster elimination. *CYP2B6**5 appeared not to alter methadone concentrations, although few subjects were studied. In general, *CYP2B6* variants had a greater influence on S- than R-methadone, and oral versus IV methadone due to first-pass metabolism. These results confirm the hypothesis that *CYP2B6**6 carriers have higher plasma methadone concentrations and reduced metabolism and clearance, and that other *CYP2B6* variants can also affect methadone disposition.

Allelic influences on methadone concentrations were caused by differences in clearance. Methadone systemic clearance comprises hepatic (metabolic) clearance and renal clearance, but depends primarily on metabolism, evidenced by correlations between systemic clearance and N-demethylation, as observed previously.^{14,33} Methadone systemic clearance was less in *CYP2B6**6 carriers, particularly homozygotes, and apparently greater in *CYP2B6**4 carriers. These differences were due specifically to altered hepatic clearance, and in turn, altered N-demethylation (EDDP/methadone plasma AUC ratios, EDDP formation clearance). Methadone N-demethylation was significantly less in *CYP2B6**6 carriers, particularly homozygotes, and apparently greater in *CYP2B6**4 carriers, compared with *CYP2B6**1/*1 wild-types. In contrast, renal elimination of unchanged methadone did not explain *CYP2B6* genotype-dependent differences in systemic clearance or plasma concentrations. Thus metabolism explains *CYP2B6* genetic influences on methadone clearance.

CYP2B6 polymorphic differences in methadone clearance *in vivo* are fully congruent with previous *in vitro* observations. Methadone N-demethylation by expressed CYP2B6.6 was significantly less than by wild-type CYP2B6.1, and, liver microsomes from *CYP2B6**6 carriers had diminished metabolism.²⁷ Conversely, CYP2B6.4 showed greater methadone N-demethylation, with CYP2B6.5 comparable to CYP2B6.1.³⁴ For methadone, at least for the alleles evaluated, metabolism and clearance *in vivo* parallels N-demethylation by CYP2B6 variants *in vitro*. *In vitro* metabolism by other CYP2B6 variants may have utility to predict *in vivo* methadone clearance. For example, metabolism by rare but important *CYP2B6* variants, such as CYP2B6.18 (which does not metabolize methadone),³⁴ may forecast clinically significant pharmacogenetic effects.

The influence of specific SNPs merits attention. *CYP2B6**4 (785A>G, K262R) carriers had apparently increased methadone metabolism and clearance *in vivo* and CYP2B6.4 had significantly higher rates of N-demethylation *in vitro*.³⁴ In contrast, *CYP2B6**6 (516G>T,

Q172H; 785A>G, K262R) carriers had diminished methadone metabolism and clearance *in vivo*, and reduced hepatic CYP2B6 protein expression and metabolism *in vitro*.²⁷ The influence of a second SNP (516G>T) in addition to 785A>G, on methadone metabolism, together conferring poor vs extensive metabolizer phenotype, is notable, and similar to *CYP2B6**6 effects on efavirenz and bupropion metabolism.³⁵⁻³⁷ Carriers of only 516G>T (*CYP2B6**9) were not evaluated in this investigation, but *in vitro* methadone N-demethylation by CYP2B6.9 was less than by CYP2B6.1,³⁴ consistent with other substrates.³⁸ The 516G>T SNP alone is credited with diminished metabolic activity of CYP2B6.6 and CYP2B6.9.³⁸ Other variant *CYP2B6* alleles with 516G>T include *CYP2B6**13, *19, *20, *26, *29, *34, *36, *37 and *38.¹⁷ The influence of these variants on methadone metabolism and clearance, *in vitro* or *in vivo*, is unknown. Interestingly, *CYP2B6**4/*6 compound heterozygotes had increased methadone metabolism and clearance, similar to a *CYP2B6**1/*4 subject. Thus a single *CYP2B6**4 extensive metabolizer allele overcame a *CYP2B6**6 poor metabolizer allele in the *CYP2B6**4/*6 haplotype. Overall, *in vivo* methadone N-demethylation in carriers of variant *CYP2B6* alleles parallels *in vitro* metabolism by their encoded CYP2B6 protein variants.

CYP2B6 genetic influence on methadone metabolism and clearance further highlights and reinforces CYP2B6 as the predominant CYP responsible for clinical methadone elimination. For many years, CYP3A4 was assumed to be responsible *in vivo*, because CYP3A4 was initially identified as catalyzing metabolism *in vitro*, and, by extrapolation, was proffered in numerous publications and clinical guidelines as responsible for methadone disposition *in vivo*.^{21,39-43} Nonetheless, it is now established, after recognizing CYP2B6 as a major catalyst of methadone metabolism *in vitro*,^{28,44-47} and from numerous clinical drug interaction studies, that CYP2B6, not CYP3A4, is the principle determinant of methadone elimination.^{3,14-16} Specifically, neither CYP3A induction⁴⁸ nor strong inhibition^{13,14,28,29,31} altered methadone N-demethylation or clearance, while CYP2B6 induction^{13,28,32,46} or inhibition^{15,49} did correspondingly modulate methadone elimination. *CYP2B6* allelic influences on plasma R/S methadone ratios further demonstrate the role of CYP2B6. CYP2B6 metabolizes methadone stereoselectively,^{28,44-47} with time-dependent increases in the R/S ratio. Drug interactions which increase CYP2B6 activity accentuate the increase,^{13,14,28,32,46,50} while those which inhibit CYP2B6 diminish the increase.¹⁵ Conversely, inhibiting CYP3A, which metabolizes methadone non-stereoselectively, had no effect.²⁹⁻³¹ In this investigation, *CYP2B6* variants with greater (*CYP2B6**4) or diminished (*CYP2B6**6) activity amplified or lessened, respectively, the time-dependent increase in R/S ratio. Together, therefore, *CYP2B6* pharmacogenetics and drug interaction studies further substantiate CYP2B6 as the major determinant of clinical methadone metabolism, clearance, and plasma concentrations.

These findings have therapeutic implications. First, they provide a mechanistic explanation for previous clinical associations between *CYP2B6**6/*6 genotype and 2-fold higher (dose-adjusted) single peak and trough plasma concentration of R-, S- and/or RS-methadone.^{21,22} or lower methadone dose requirements.²⁴⁻²⁶ By actually measuring methadone plasma concentrations throughout the elimination period, and formally determining methadone systemic clearance, hepatic clearance, metabolism, and renal clearance, these prior

observations can now be explained by diminished methadone N-demethylation and clearance in *CYP2B6**6 carriers. Second, while *CYP2B6* polymorphisms affected S- more than R-methadone, results for both enantiomers are important, because S-methadone affects the metabolism of R-methadone,⁴⁵ and, while R-methadone is more active at μ opioid receptors, both enantiomers have N-methyl-D-aspartate receptor activity. Third, this investigation newly links *CYP2B6**4 with increased methadone metabolism and clearance, and decreased methadone concentrations, identifying an apparent extensive metabolizer phenotype. Such individuals may have increased dose requirements for pain control (or have subtherapeutic plasma concentrations at standard doses used for addiction therapy and hence at risk for withdrawal), and particular susceptibility to *CYP2B6* inhibitory drug interactions. Fourth, these results identify a genetic etiology for the well-known but previously unexplained interindividual variability in methadone elimination (and dose requirements). Thus, *CYP2B6* polymorphisms contribute to constitutive (not influenced by drug interactions) heterogeneity in methadone metabolism and clearance, and thus, both *CYP2B6* pharmacogenetics and *CYP2B6* drug interactions¹¹ influence methadone interindividual variability. Fifth, *CYP2B6* polymorphisms have greater consequence for oral (pain and addiction therapy) than intravenous (intraoperative, where *CYP2B6* genetics appear inconsequential) methadone, and for repeat (steady-state) versus single-dosing. The common occurrence of *CYP2B6**6 (allele frequency 33-50% in Africans and African-Americans, 10-21% in Asians, 14-27% in Caucasians, and 62% in Papua New Guineans),¹⁷ makes this variant clinically significant for oral methadone. Similarly, other alleles, such as loss of function *CYP2B6**18 (4-11% frequency) may also be relevant, as poor metabolizers may be at risk for methadone toxicity. Lastly, this investigation attends individualized therapy, and engenders the question whether *CYP2B6* genotyping and genetically-guided methadone dosing may have value. The relevant example is the antiretroviral drug and *CYP2B6* substrate efavirenz, with genetically-based interindividual variability in metabolism, clearance, plasma concentrations and exposure, influencing both efficacy and toxicity.^{17,51} *CYP2B6**6 carriers have deficient efavirenz metabolism, higher plasma concentrations, and a greater incidence of toxicity.^{17,51} Efavirenz dose-reduction is recommended in heterozygous and homozygous *CYP2B6* 516G>T carriers.⁵² By analogy, the question arises whether methadone dose-reduction would be appropriate in 516G>T carriers, and whether *CYP2B6**6 genetically-guided dosing would reduce methadone interindividual (genetically-dependent) variability in plasma concentrations, side effects and toxicity, particularly death due to overdose, principally when used for pain therapy. Indeed, in methadone-related fatalities, there was a significant association between high methadone concentrations and the *CYP2B6**6 allele.⁵³ More broadly, since several other loss of function alleles (e.g. *CYP2B6**18, *20, *27) have been associated with increased efavirenz concentrations,⁵¹ these too may result in methadone poor metabolizer status, and put patients at risk for toxicity.

This investigation has limitations. Healthy volunteers were studied to eliminate potential confounding by disease or drug interactions. Nevertheless, this meant evaluating a single methadone dose, precluding formal assessment of metabolism and clearance and *CYP2B6* variant effects at steady-state. Because methadone causes CYP upregulation and 2-fold autoinduction of its own clearance with repeat dosing,^{54,55} and *CYP2B6* activity is less

inducible in *CYP2B6**6 vs *CYP2B6**1 carriers,⁵⁶⁻⁵⁸ differences in methadone clearance in *CYP2B6**6 carriers would be expectedly greater at steady-state than with a single dose. Furthermore, R- and S-methadone mutually influence each other's metabolism,⁴⁵ an effect likely more apparent at steady-state, and further amplifying *CYP2B6* genotypic differences. These may explain why differences between *CYP2B6**6 vs *CYP2B6**1 carriers at steady-state were numerically greater^{21,22} than those in the present single-dose study. These steady-state considerations merit clinical verification. A second limitation is the small number of other allelic variants studied (*4, *5), and the small sample sizes in these minor variant groups, as this was not a primary study objective, their enrollment was incidental, and the allele frequencies are rare. Results observed with these genotypes agree well with in vitro data on methadone metabolism by CYP2B6.4 and CYP2B6.5.³⁴ Nevertheless, these clinical results should be interpreted conservatively and verified with larger groups. Lastly, lack of bioavailability differences in *CYP2B6**6 carriers despite different oral clearances is unexplained.

In summary, in healthy volunteers hetero- or homozygous for the *CYP2B6**4, *CYP2B6**5, or *CYP2B6**6 alleles, intravenous and oral methadone plasma concentrations were greater in *CYP2B6**6 carriers and lower in *CYP2B6**4 carriers, and relatively unchanged in *CYP2B6**5 carriers compared with wild-type *CYP2B6**1/*1 subjects. *CYP2B6* genotype-related differences in plasma exposure were due to alterations in methadone systemic clearance, caused in turn by differences in methadone N-demethylation. Genotypic influence was greater for S- than R-methadone and oral versus intravenous methadone. These results provide a mechanistic understanding for interindividual variability in methadone elimination, and may have clinical implications for genetically-based improvements in methadone dosing, effectiveness, and toxicity.

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Figure 1.

Influence of *CYP2B6* genotype on the disposition and metabolism of oral methadone. Subjects received 11.0 mg oral methadone HCl (9.9 mg free base). Shown are plasma concentrations of (A) R-methadone, (B) S-methadone, (C) R-EDDP, and (D) S-EDDP and (E) R/S-methadone concentration ratios. Each data point is the mean $\pm$ SD. Some SD values are omitted for clarity. Genotype cohorts were *CYP2B6**1/*1 (n=21), *CYP2B6**1/*6 (n=20), *CYP2B6**6/*6 (n=17) and *CYP2B6**4/X (n=4, with results for one *CYP2B6**1/*4 and three *CYP2B6**4/*6 subjects combined). EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

Figure 2.

Influence of *CYP2B6* genotype on the disposition and metabolism of intravenous methadone. Subjects received 6.0 mg intravenous methadone HCl (5.4 mg free base). Shown are plasma concentrations of (A) R-methadone, (B) S-methadone, (C) R-EDDP, and (D) S-EDDP. Each data point is the mean $\pm$ SD. Some SD values are omitted for clarity. Genotype cohorts were *CYP2B6**1/*1 (n=21), *CYP2B6**1/*6 (n=20), *CYP2B6**6/*6 (n=17) and *CYP2B6**4/X (n=4, with results for one *CYP2B6**1/*4 and three *CYP2B6**4/*6 subjects combined). EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

Figure 3.

Influence of minor *CYP2B6* alleles on the disposition and metabolism of intravenous and oral methadone. Subjects received 6.0 mg intravenous methadone HCl and 11.0 mg oral methadone HCl. Shown are plasma concentrations of (A) R-methadone, (B) S-methadone, (C) R-methadone, and (D) S-methadone after intravenous (A,B) and oral (C,D) dosing. Each data point is the mean. Genotype cohorts were *CYP2B6**1/*1 (n=21), *CYP2B6**1/*4 (n=1), *CYP2B6**4/*6 (n=3) and *CYP2B6**5/*5 (n=2).

Figure 4.

Influence of *CYP2B6**6 genotype on methadone clearance. Shown are the systemic clearances for intravenous (A) R-methadone and (B) S-methadone, and the apparent oral clearances for oral (C) R-methadone and (D) S-methadone, as box plots (solid line within the box is the median, dash line within the box is the mean, box boundaries are the 25th and 75th percentiles, error bars are the 10th and 90th percentiles, and individual points are outliers). *Significantly different from wild-type (*CYP2B6**1/*1), $p < 0.05$.

Figure 5.

Influence of *CYP2B6**6 genotype on methadone metabolism. Shown is the plasma concentration vs time AUC ratio for EDDP/methadone for intravenous (A) R-methadone and (B) S-methadone, and oral (C) R-methadone and (D) S-methadone, and the EDDP formation clearance for intravenous (E) R-methadone and (F) S-methadone, and oral (G) R-methadone and (H) S-methadone. Results are shown as box plots (solid line within the box is the median, dash line within the box is the mean, box boundaries are the 25th and 75th percentiles, error bars are the 10th and 90th percentiles, and individual points are outliers). *Significantly different from wild-type (*CYP2B6**1/*1), $p < 0.05$. EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

Table 1

Subject demographics

<i>CYP2B6</i> Genotype	Sex (M:F)	Age (yr)	Weight (kg)	Caucasian	African American	Asian	Other/Unknown
*1/*1	9:12	28 ± 7	72 ± 14	13	1	7	
*1/*6	14:6	28 ± 8	78 ± 11	14	3	2	1
*6/*6	7:10	32 ± 9	71 ± 13	9	6	1	1
*1/*4	1:0	22	68	1			
*4/*6	1:2	29 ± 2	68 ± 15	3			
*5/*5	2:0	28 ± 1	84 ± 0	2			
Total	34:30	29 ± 8	74 ± 13	42	10	10	2

Table 2
Racial differences in oral methadone clearance and metabolism

	Caucasian (n=44)		African-American (n=10)	
	R-methadone	S-methadone	R-methadone	S-methadone
Apparent oral clearance (ml/kg/min)	2.4 ± 1.8	2.3 ± 2.3	1.4 ± 0.5*	1.2 ± 0.6*
plasma EDDP/methadone AUC ₀₋₉₆ ratio	0.074 ± 0.23	0.097 ± 0.045	0.058 ± 0.20*	0.072 ± 0.042*
EDDP formation clearance (ml/kg/min)	0.27 ± 0.15	0.43 ± 0.44	0.14 ± 0.08*	0.20 ± 0.13*

Genotype composition of the Caucasians was (*CYP2B6**1/*1, n=14; *CYP2B6**1/*6, n=15; *CYP2B6**6/*6, n=9; *CYP2B6**4/*X, n=4, *CYP2B6**5/*5, n=2), and the African-Americans was (*CYP2B6**1/*1, n=1; *CYP2B6**1/*6, n=3; *CYP2B6**6/*6, n=6).

AUC₀₋₉₆ = area under the plasma concentration–time curve; EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

* Significantly different from Caucasians (p<0.05).