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), and CYP2B6*5/*5 (n = 2) subjects, received single doses of IV 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. R-methadone apparent oral clearance was 25 and 35% lower in CYP2B6*1/*6 and CYP2B6*6/*6 genotypes, respectively, compared with CYP2B6*1/*1. R- and S-methadone apparent oral clearance was threefold and fourfold greater in CYP2B6*4 carriers. IV and oral R- and S-methadone metabolism was significantly lower in CYP2B6*6 carriers compared with that of CYP2B6*4 homozygotes and greater in CYP2B6*4 carriers. Methadone metabolism and clearance were lower in African Americans in part because of the CYP2B6*6 genetic polymorphism.

Conclusions: CYP2B6 polymorphisms influence methadone plasma concentrations, because of altered methadone metabolism and thus clearance. Genetic influence is greater for oral than IV methadone and S- than R-methadone. CYP2B6 pharmacogenetics explains, in part, interindividual variability in methadone elimination. CYP2B6 genetic effects on methadone metabolism and clearance may identify subjects at risk for methadone toxicity and drug interactions. (Anesthesiology 2015; 123:1142-53)

What We Already Know about This Topic

• CYP2B6 is the major determinant of clinical methadone elimination
• The allelic variant CYP2B6.6 protein deficiently metabolizes methadone in vitro but the effects of CYP2B6 gene variants on clinical methadone clearance and metabolism are unknown

What This Article Tells Us That Is New

• Compared with wild-type individuals, methadone metabolism and clearance were diminished in CYP2B6*6 carriers and increased in CYP2B6*4 carriers
• Methadone metabolism and clearance were significantly lower in African Americans because of a larger proportion of CYP2B6*6 carriers and the absence of CYP2B6*4 carriers
• These results may permit identification of individuals at risk for methadone overdose and may suggest genetically guided methadone dosing

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 M-μ-opioid receptor activity, while both enantiomers act at N-methyl-D-aspartate receptors.5 Clinical utility includes effectiveness in opioid tolerance, for pain, and for neonates through adults, with rapid onset, administration by multiple routes, high oral bioavailability, and no active metabolites. In the United States, more than 300,000 patients in opioid treatment programs receive methadone annually.6 Methadone use for pain has grown markedly. Prescriptions increased fivefold from 2000 to 2009,7 with more than 5 million annually for pain,7 the majority of which are written by primary care and nonpain physicians.8

Opioid fatalities are a growing public health problem. Specifically, with expanded use, methadone fatalities increased more than fivefold and methadone was involved in approximately one third of opioid-related overdose deaths from 1999 to 2009.7,9 In 2009, methadone accounted for only 2% of prescriptions, but 30% of prescription pain-killer deaths.7 Increased methadone mortality is attributed...
1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and some \(-\)demethylation to inactive 2-ethyl-P450 (CYP)-catalyzed N
cohorts were composed to evaluate methadone disposition.

volunteers were genotyped, and then common genotypic variants, when encountered. Healthy clearance. A secondary objective was to evaluate other less heterozy-
CYP2B6*6 metabolism. The hypothesis was that clinical methadone plasma concentrations, clearance, and polymorphism, on genetic variation, specifically variants, on methadone metabolism and clearance, is unknown. CYP2B6*6CYP2B6 in \(\text{vivo}\), or other. However, the influence of variants might influence methadone elimination CYP2B6 in vivo, or other (516G>T , 785A>G, 983T>C). Another notable, albeit less common, variant is CYP2B6*4, which causes 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 in heterozygotes and noncarriers,21-24 and methadone dose requirements were lower.24-26 However, 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 reduced the 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

The CYP2B6 gene is highly polymorphic,17 with numerous single-nucleotide polymorphisms responsible for 38 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 the 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

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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 heterozygotes or homozygotes 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 were 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 and registered (ClinicalTrials.gov Identifier: NCT01648283). All subjects provided written informed consent. Inclusion criteria were 18- to 50-yr-old normal healthy volunteers (smokers or nonsmokers) in good general health without remarkable medical conditions and within 30% of ideal body weight (body mass index < 33 kg/m²). Exclusion criteria were a history of hepatic or renal disease, use of prescription or nonprescription medications, herbasals or foods known to be metabolized by or affect the 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 by using the Gentra Puregene Blood Kit (Qiagen, USA). 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 by using the Fluidigm BioMark System (Fluidigm, USA). Primer sequences were as follows: 516G>T (rs3745274) forward: CTTGACCTGCTCTTCTTCTCTA, reverse: AGACGATGGAGCAGATGATGTG; 785A>G (rs2279343) forward: TGGGAAGACCCGTGAACC, reverse: TGGAGACGAGTGTGTCGAT; 983T>C (rs28399499) forward: TGTCCTCTTTTCTGTACAGAGAGT, reverse: GCCATGTGGGGGCAATCAC; and 1459C>T (rs3211371) forward: GTGTGTTGGCGCAAAATACC, reverse: CTTCCCTACGGCCCTTCAG. The 48 × 48 genotyping chip was primed by using the Integrated Fluidic Circuit Controller MX (Fluidigm). Samples were loaded into the sample inlets of the chips mixed with universal polymerase chain reaction master mix (Life Technologies, USA), 20× GT loading reagent (Fluidigm), and AmpliTaq Gold Polymerase (Life Technologies) at 100 ng/μL. The 40× TaqMan genotyping assays (Life Technologies) were loaded with 2× assay loading reagent and 6-carboxyl-X-rhodamine (Life Technologies) in the assay inlets, six replicates per assay. The samples and assays were loaded in the chips by using the Integrated Fluidic Circuit Controller MX. The chip was cycled by using the Fluidigm BioMark. Results were loaded into Fluidigm SNP Genotyping Analysis Software for further analysis. Analysis of these SNPs permitted the detection of the CYP2B6*1, CYP2B6*4 (785A>G), CYP2B6*5 (1459C>T), CYP2B6*6 (516G>T, 785A>G), CYP2B6*7 (516G>T, 785A>G, 1459C>T), CYP2B6*9 (516G>T), CYP2B6*16 (785A>G, 983T>C), and CYP2B6*18 (983T>C) alleles.

Genotyping results were then used to invite subject participation and create target cohorts of 20 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 subjects per group. In addition, subjects of other rare genotypes coincidentally identified were also studied. A total of 64 subjects (34 men, 30 women; 42 Caucasians, 10 African Americans, 10 Asians, and 2 other/unknown) of age 29 ± 8 yr and weight 74 ± 13 kg were studied. Detailed demographic data are provided in table 1.

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

Study design was a single-center, open-label, and single-session protocol. Methadone disposition was assessed by simultaneously administering IV and oral methadone. 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 blood sampling and IV drug administration. Subjects received a standard breakfast and lunch 2 and 4 h after methadone administration, respectively, and free access to food and water thereafter. Venous blood was sampled for 96 h after methadone administration, and plasma was stored at −80°C. Continuous urine samples were collected at 24, 48, 72, and 96 h and stored at −80°C. Nausea and/or vomiting was treated with ondansetron (4 mg IV or 8 mg orally) as needed. Subjects were monitored by 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. Interday coefficients of variation for methadone and EDDP were 6 to 13% in plasma and 3 to 10% in urine.

### Table 1. Subject Demographics

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<th>CYP2B6 Genotype</th>
<th>Sex (M:F)</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
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<th>African</th>
<th>American</th>
<th>Asian</th>
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<tr>
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<td>32±9</td>
<td>71±13</td>
<td>9</td>
<td>6</td>
<td>1</td>
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<tr>
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<td>68±15</td>
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<td>29±8</td>
<td>74±13</td>
<td>42</td>
<td>10</td>
<td>10</td>
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</table>

### Data and Statistical Analysis

Pharmacokinetic data were analyzed by using noncompartmental methods (Phoenix, Pharsight Corp, USA), assuming complete absorption, as described previously. Results are reported as the arithmetic mean ± SD. The primary outcome measure was methadone metabolism, measured as plasma EDDP/methadone area under the concentration–time curve (AUC\(_{\infty–96} \)) ratio and EDDP formation clearance. Secondary outcomes included methadone peak plasma concentration, exposure (plasma AUC\(_{\infty} \)), 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 by using one-way analysis of variance followed by the Student–Newman–Keuls test for multiple comparisons (Sigmplot 12.5, Systat Software, Inc., USA). Nonnormal data were log transformed for analysis but reported as the nontransformed results. Racial groups were compared by using Student’s t test. Statistical significance was assigned at \( P < 0.05 \). Formal comparison of other CYP2B6 allelic variants with CYP2B6*1/*1 subjects was not performed because of the small subject numbers studied. Relationships between methadone clearance and metabolism were evaluated by 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, and CYP2B6*18 0.016) are consistent with the previous reports. 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 (fig. 1) and IV (fig. 2) methadone, for the three major genotype groups (CYP2B6*1/*1, CYP2B6*1/*6, and 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 IV dosing and for S- rather than R-methadone. For oral methadone, average plasma exposure (area under the curve, AUC$_\infty$, ng/ml-h) in CYP2B6*1/*1, CYP2B6*1/*6, and CYP2B6*6/*6 cohorts was 620 ± 230, 734 ± 245, and 1,242 ± 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$_\infty$ for CYP2B6*4/X subjects was 155 ± 45 and 177 ± 48 for S- and R-methadone, respectively.

IV and oral methadone plasma concentrations in carriers of minor CYP2B6 allelic variants are shown in figure 3. CYP2B6*5 homozygote concentrations resembled those of CYP2B6*1 homozygotes, whereas CYP2B6*4/*4 and CYP2B6*4/*6 subjects had lower concentrations, particularly with oral methadone.

Fig. 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, (D) S-EDDP, and (E) R/S-methadone concentration ratios. Each data point is the mean ± 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.
Methadone disposition was stereoselective, with greater initial exposure to S-methadone and a time-dependent increase in the plasma methadone R/S concentration ratio (figs. 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 IV drug, S-methadone systemic clearance (ml kg⁻¹ min⁻¹) 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; fig. 4). R-methadone clearances in CYP2B6*6 carriers were not significantly different from CYP2B6*1/*1 subjects. Hepatic clearance (ml kg⁻¹ min⁻¹) was significantly less in CYP2B6*6/*6 compared with that of 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 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; data 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/*1 homozygotes (2.3 ± 1.5; fig. 4). R-methadone apparent oral clearance was also significantly less in CYP2B6*6 than in CYP2B6*1/*1 homozygotes (1.6 ± 0.7 vs. 2.4 ± 1.2, respectively). In contrast, 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 in CYP2B6*4/*X subjects than in 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 ± 18%, respectively) or R-methadone (75 ± 20%, 80 ± 13%, and 84 ± 16%, respectively) but was numerically lower in CYP2B6*4/*X subjects (39 ± 21% and 34 ± 19% for R- and S-methadone, respectively, data 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; data not shown). Methadone N-demethylation, evaluated from both the plasma EDDP:methadone AUC ratio and EDDP formation clearance, for both IV and oral methadone and both enantiomers, was significantly lower in CYP2B6*6 carriers compared with that of CYP2B6*1 homozygotes (fig. 5). Conversely, N-demethylation was numerically greater in CYP2B6*4/*X than in CYP2B6*1/*1 subjects. For example, for IV methadone, in CYP2B6*4/*X and CYP2B6*1/*1...
Subjects, R-EDDP formation clearances (ml kg⁻¹ min⁻¹) 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, respectively. Results were comparable for oral methadone. IV methadone renal clearance was not affected by CYP2B6 genotype (data 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. 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 CYP2B*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 the 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, whereas 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-methadone than R-methadone, and oral versus IV methadone because of 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. Methadone systemic clearance was less in CYP2B6*6 carriers, particularly homozygotes, and apparently greater in CYP2B6*4 carriers. These differences were specifically because of altered hepatic clearance and, in turn, altered N-demethylation (EDDP:methadone plasma AUC ratios and EDDP formation clearance). Methadone N-demethylation was significantly less in CYP2B6*6 carriers, particularly homozygotes, and apparently greater in CYP2B6*4 carriers, compared with...
that of wild-type CYP2B6*1/*1. 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 than CYP2B6.1, while CYPs 2B6.5 and 2B6.1 were comparable.34 For methadone, at least for the alleles evaluated, metabolism and clearance in vivo parallel 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 versus 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, CYP2B6*19, CYP2B6*20, CYP2B6*26, CYP2B6*29, CYP2B6*34, CYP2B6*36, CYP2B6*37, and CYP2B6*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

Fig. 4. Influence of CYP2B6*6 genotype on methadone clearance. Shown are the systemic clearances for IV (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 represents the median, dashed line within the box represents 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. CL/F = apparent oral clearance.
Fig. 5. Influence of CYP2B6*6 genotype on methadone metabolism. Shown is the plasma concentration versus time AUC ratio for EDPD/methadone for IV (A) R-methadone and (B) S-methadone and oral (C) R-methadone and (D) S-methadone, and the EDPD formation clearance for IV (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 represents the median, dashed line within the box represents 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. AUC = area under the curve; EDPD = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.
Table 2. Racial Differences in Oral Methadone Clearance and Metabolism

<table>
<thead>
<tr>
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<th>Caucasian (n = 44)</th>
<th>African American (n = 10)</th>
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<tbody>
<tr>
<td></td>
<td>R-Methadone</td>
<td>S-Methadone</td>
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<tr>
<td>Apparent oral clearance (ml kg(^{-1}) min(^{-1}))</td>
<td>2.4 ± 1.8</td>
<td>2.3 ± 2.3</td>
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<tr>
<td>Plasma EDDP:methadone AUC(_{0-96}) ratio</td>
<td>0.074 ± 0.23</td>
<td>0.097 ± 0.045</td>
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<tr>
<td>EDDP formation clearance (ml kg(^{-1}) min(^{-1}))</td>
<td>0.27 ± 0.15</td>
<td>0.43 ± 0.44</td>
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<tr>
<td></td>
<td>R-Methadone</td>
<td>S-Methadone</td>
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<tr>
<td></td>
<td>1.4 ± 0.5*</td>
<td>1.2 ± 0.6*</td>
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Genotype composition of the Caucasian was as follows: CYP2B6*1/*1, n = 14; CYP2B6*1/*6, n = 15; CYP2B6*6/*6, n = 9; CYP2B6*4/*X, n = 4; and CYP2B6*5/*5, n = 2, and the African Americans was as follows: CYP2B6*1/*1, n = 1; CYP2B6*1/*6, n = 3; and CYP2B6*6/*6, n = 6. * Significantly different from Caucasians (P < 0.05).

AUC\(_{0-96}\) = area under the plasma concentration–time curve; EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine.

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. However, it is now established, after recognizing CYP2B6 as a major catalyst of methadone metabolism in vitro, and from numerous clinical drug interaction studies, that CYP2B6, not CYP3A4, is the principle determinant of methadone elimination. Specifically, neither CYP3A induction nor strong inhibition altered methadone N-demethylation or clearance, whereas CYP2B6 induction or inhibition did correspondingly modulate methadone elimination. CYP2B6 allelic influences on plasma R/S methadone ratios further demonstrate the role of CYP2B6. CYP2B6 metabolizes methadone stereoselectively, and the time-dependent increases in the R:S ratio. Drug interactions that increase CYP2B6 activity accentuate the increase, whereas those that inhibit CYP2B6 diminish the increase. Conversely, inhibiting CYP3A, which metabolizes methadone nonstereoselectively, had no effect. In this investigation, CYP2B6 variants with greater (CYP2B6*4) or diminished (CYP2B6*6) activity amplified or reduced, 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*4/*6 genotype and two-fold higher (dose-adjusted) single peak and trough plasma concentration of R-, S- and/or RS-methadone or lower methadone dose requirements. By actually measuring methadone plasma concentrations throughout the elimination period, and formally determining methadone systemic clearance, hepatic clearance, metabolism, and renal clearance, these previous observations can now be explained by diminished methadone N-demethylation and clearance and in CYP2B6*6 carriers. Second, although CYP2B6 polymorphisms affected S-methadone more than R-methadone, results for both enantiomers are important, because S-methadone affects the metabolism of R-methadone, and although R-methadone is more active at \(\mu\)-opioid receptors, both enantiomers have \(N\)-methyl-\(\delta\)-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 interactions influence methadone interindividual variability. Fifth, CYP2B6 polymorphisms have greater consequence for oral methadone (pain and addiction therapy) than IV (intraoperative, where CYP2B6 genetics appear inconsequential) methadone and for repeat (steady state) versus single dosing. The common occurrence of CYP2B6*6 (allele frequency: 33 to 50% in Africans and African Americans, 10 to 21% in Asians, 14 to 27% in Caucasians, and 62% in Papua New Guineans) makes this variant clinically significant for oral methadone. Similarly, other alleles, such as loss of function CYP2B6*18 (4 to 11% frequency) may also be relevant, because poor metabolizers may be at risk for methadone toxicity. Finally, 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. CYP2B6
have been associated with increased efavirenz concentrations, and a greater incidence of toxicity. 

Efavirenz dose reduction is recommended in heterozygous and homozygous CYP2B6 516G>T carriers. 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 inter-individual (genetically dependent) variability in plasma concentrations, side effects, and toxicity, particularly death because of 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. More broadly, because several other loss-of-function alleles (e.g., CYP2B6*18, *20, *27) have been associated with increased efavirenz concentrations, 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. However, 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 up-regulation and twofold autoinduction of its own clearance with repeat dosing, and CYP2B6 activity is less inducible in CYP2B6*6 versus CYP2B6*1 carriers, 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, an effect likely more apparent at steady state, and further amplifying CYP2B6 genotypic differences. These may explain why differences between CYP2B6*6 versus CYP2B6*1 carriers at steady state were numerically greater 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, because 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. However, these clinical results should be interpreted conservatively and verified with larger groups. Finally, the lack of bioavailability differences in CYP2B6*6 carriers despite different oral clearances is unexplained.

In summary, in healthy volunteers, heterozygous or homozygous for the CYP2B6*4, CYP2B6*5, or CYP2B6*6 alleles, IV and oral methadone plasma concentrations were greater in CYP2B6*6 carriers, lower in CYP2B6*4 carriers, and relatively unchanged in CYP2B6*5 carriers compared with that of 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-methadone than R-methadone and oral versus IV 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.

Acknowledgments

The authors thank Jennifer Parchomski, R.N., Department of Anesthesiology, Washington University in St. Louis, St. Louis, Missouri, for her excellent clinical research assistance, and Chris Sawyer and Richard Head, Ph.D., Genome Technology Access Center and Department of Genetics at Washington University in St. Louis School of Medicine, St. Louis, Missouri, for help with the conduct and interpretation of the CYP2B6 genomic analysis.

This investigation was supported by National Institutes of Health (Bethesda, Maryland) grants R01-DA14211, R01-DA25931, and K24-DA00417 (to Dr. Kharasch).

The Washington University Institute of Clinical and Translational Sciences is supported by grant UL1TR000448 from the National Center for Advancing Translational Sciences of the National Institutes of Health. The Genome Technology Access Center is partially supported by National Cancer Institute Cancer Center Support grant P30CA91842 to the Siteman Cancer Center (St. Louis, Missouri) and by the Washington University Institute of Clinical and Translational Sciences (UL1TR000448).

Competing Interests

The authors declare no competing interests.

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