7-1-2018

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Effects of cytochrome P450 single nucleotide polymorphisms on methadone metabolism and pharmacodynamics

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Abstract

Methadone is a synthetic, long-acting opioid with a single chiral center forming two enantiomers, (R)-methadone and (S)-methadone, each having specific pharmacological actions. Concentrations of (R)- and (S)-methadone above therapeutic levels have the ability to cause serious, life-threatening, and fatal side effects. This toxicity can be due in part to the pharmacogenetics of an individual, which influences the pharmacokinetic and pharmacodynamic properties of the drug. Methadone is primarily metabolized in the liver by cytochrome P450 (CYP) enzymes, predominately by CYP2B6, followed by CYP3A4, 2C19, 2D6, and to a lesser extent, CYP2C18, 3A7, 2C8, 2C9, 3A5, and 1A2. Single nucleotide polymorphisms (SNPs) located within CYPs have the potential to play an important role in altering methadone metabolism and pharmacodynamics. Several SNPs in the *CYP2B6, 3A4, 2C19, 2D6,* and 3A5 genes result in increases in methadone plasma concentrations, decreased N-demethylation, and decreased methadone clearance. In particular, carriers of *CYP2B6*6/*6* may have a greater risk for detrimental adverse effects, as methadone metabolism and clearance are diminished in these individuals. *CYP2B6*4*, on the other hand, has been observed to decrease plasma concentrations of methadone due to increased methadone clearance. The involvement, contribution, and understanding the role of SNPs in *CYP2B6*, and other CYP genes, in methadone metabolism can improve the therapeutic uses of methadone in patient outcome and the development of personalized medicine.

Keywords

Methadone metabolism; Single nucleotide polymorphism (SNP); Cytochrome P450 (CYP); Pharmacogenetics; Genetic variant

1. Methadone pharmacology

Shortly after methadone was introduced in the United States, the Food and Drug Administration (FDA) approved its use as an analgesic and antitussive agent [1]. During the 1960s, evidence for the usefulness of methadone in the treatment of and maintenance

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therapy for narcotic addiction was growing [2,3]. A decade later, the FDA expanded the approval for physicians and hospital pharmacies to dispense methadone for treating opiate addiction in treatment programs only [1]. Enrollment in a methadone maintenance treatment (MMT) program also has the potential to be a public health strategy for decreasing the transmission of infectious diseases (e.g. HIV/AIDS, hepatitis C, or other blood-borne pathogens) by reducing the number of opioid users reusing contaminated needles [3–5].

Methadone is a synthetic, long-acting opioid with a single chiral center that forms two enantiomers, R-(−)-methadone (*levo*-methadone) and S-(+)-methadone (*dextro*-methadone) [6,7]. Both enantiomers have specific pharmacodynamic properties and pharmacological actions. (R)-Methadone is a μ-opioid receptor (MOR) agonist that mimics the body's natural endogenous opioids, endorphins and enkephalins.Binding of (R)-methadone to the MOR mediates therapeutic effects with the release of other neurotransmitters, including acetylcholine, dopa-mine, norepinephrine, and substance P [6]. The effects propagated by this interaction yields reduction and elimination of drug cravings and withdrawal signs and symptoms of narcotics, analgesia, sedation, nausea, vomiting, constipation, miosis, antitussive effects, hypotension, mild bradycardia, and tolerance and dependence [8–11].

In the United States, methadone is clinically available only as a racemic formulation comprised of both (R)- and (S)-methadone enantiomers [12–14]. Methadone is currently used as: (1) an approved opioid substitution therapeutic agent to suppress opioid-abstinence syndrome in opiate abuse and addiction [15–19]; (2) a second-line opioid in alleviating cancer pain and chronic non-malignant pain, such as back, joint, or neuropathic pain and other forms of severe pain [20–23]; and (3) a first-line treatment in patients with both opioid dependence and chronic pain [24].

Methadone has contributed to a significant portion of opioid-related overdose deaths. However, this outcome is not attributed to the use of methadone for opioid dependence, rather from prescriptions of methadone for pain management [25]. Reports from the Centers for Disease Control and Prevention (CDC) indicate a 15.6% increase in opioid death rates from 2014 to 2015. Conversely, methadone death rates decreased by 9.1% due to factors such as efforts to reduce methadone therapy for pain, warning labels, clinical guidelines, and limiting high dose formulations [26]. However, death rates continued to increase through 2014 resulting from methadone overdosing in individuals between the ages of 55–64 years [25].

Concentrations of (R)- and (S)-methadone above therapeutic levels have negative and detrimental side effects. Elevated (R)-methadone levels may depress ventilation, inducing respiratory depression, by acting on the MORs expressed on respiratory centers in the brainstem [27–29]. Conversely, an increase in (S)-methadone disposition causes cardiotoxicity through the blockage of the voltage-gated potassium channel of the human ether-a-go-go related gene (hERG), subsequently prolonging the QT interval of an electrocardiogram leading to torsades de pointes. (S)-Methadone is 3.5 times more potent than (R)-methadone in blocking the hERG channel, thus attributing the stereoselectivity of methadone to cardiotoxicity [30–33].

Methadone is primarily eliminated via hepatic metabolism by cytochrome P450 (CYP) enzymes through oxidative biotransformation. Methadone undergoes stereoselective N-demethylation followed by spontaneous cyclization to form the principle, inactive metabolite 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) (Fig. 1) [34–39].Methadone has a broad half-life of elimination ranging from 5 to 130 h, with an average of 22 h [40]. This extreme inconsistency in the elimination half-life exemplifies the large variability methadone possesses in its pharmacokinetics between individuals. Because of the inter-individual variation, it is difficult to predict an individual's peak plasma levels, which may result in some individuals having a higher than normal plasma level that approaches the toxic or even fatal drug concentration. Co-medication, age, gender, health, weight, and ethnicities can all be factors that contribute to this effect.

Genetic variation occurs within and among populations, leading to polymorphisms. Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation. A SNP is a variation of a single nucleotide at a specific position in the genome, some of which may contribute to changes in a gene, either in the coding (exons) or non- coding (introns) regions, or the regions between genes. A genetic variation within CYP genes responsible for the metabolism of methadone may be causal for altered function, which may result in methadone being rapidly eliminated from the body, thus preventing the drug from reaching therapeutic levels. Alternatively, the genetic variation may prolong elimination from the body, consequently, intensifying analgesic and detrimental adverse effects [41,42]. The purpose of this review is to focus on the effects of SNPs of the various CYPs that metabolize methadone.

2. Methadone metabolism

Eleven metabolites from methadone metabolism have been isolated and identified in urine and feces in humans [43]. Methadone predominantly forms the pharmacologically inactive pyrrolidine metabolites EDDP and 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP). Methadol and normethadol are active analgesic metabolites of methadone, although this pathway is relatively minor [44,45]. To a lesser extent, methadone, normethadol, EDDP, and EMDP are further hydroxylated to form inactive *p*-hydroxy metabolites. Another minor pathway yields the inactive metabolites 4-dimethylamino-2,2-diphenylvaleric acid, 4-methylamino-2,2-diphenylvaleric acid, and 1,5-dimethyl-3,3-diphenyl-2-pyrrolidone (Fig. 1) [43–45].

The CYP enzymes involved in the formation of methadone meta-bolites in humans are CYP2B6, 3A4, 2C19, 2D6, and to a lesser extent, CYP2C18, 3A7, 2C8, 2C9, 3A5, and 1A2 [46]. These CYPs, however, metabolize methadone in a stereoselective manner, where CYP2C19, 3A7, and 2C8 preferentially metabolize (R)-methadone, CYP2B6, 2D6, and 2C18 primarily metabolize (S)-methadone, and CYP3A4 demonstrates no stereoselectivity in methadone metabolism [46,47]. Methadone is N-demethylated to an intermediate compound, normethadone, which is then spontaneously cyclized to form EDDP. A second N-demethylation reaction occurs on EDDP to form EMDP [35–37,44,48]. An alternate metabolic pathway in the formation of EMDP is a CYP mediated N-demethlyation of normethadone to form dinormethadone, which is then spontaneously cyclized to EMDP

[47]. In a minor bio-transformation pathway, the keto group of methadone is reduced by alcohol dehydrogenase to form methadol, which can be N-demethylated to form normethadol. The inactive (S)-methadone produces active metabolites of α-l-methadol and α-l-normethadol, which have analgesic properties comparable to (R)-methadone. (R)-Methadone, on the other-hand, is metabolized to biologically inactive metabolites of d-methadol and d-normethadol [44,45,47].

Methadone's long ranging half-life can be partially attributed to the extreme interindividual pharmacokinetics and its stereoselective metabolism [49,50]. (S)-Methadone exhibits a significantly shorter elimination half-life than (R)-methadone [48,51]. Inherited polymorphisms in CYPs have the potential to affect the metabolic rate of methadone in a stereoselective manner. The following sections will discuss the role of CYP SNPs in methadone metabolism and its consequences on biological activity.

3. Cytochrome P450 (CYP)

There are approximately 57 active human CYP genes grouped into 18 families and 44 subfamilies. Only 12 CYP enzymes, which belong to the CYP1, CYP2, or CYP3 families, are of major importance and responsible for the metabolism of more than 95% of therapeutic drugs [52–54]. The involvement and contribution of SNPs is inadequately understood in drug biotransformation, but are known to play a vital role in the differences in patient outcome and the development of personalized medicine [55]. SNPs can cause a loss of function mutation, in which the splicing, expression, or transcription of the CYP gene is decreased or the protein structure is altered. Alternatively, gain of function mutations may also occur, resulting in an increased substrate turnover due to an increased number of functional gene copies, as well as variations in the promotor or amino acids [54,56,57]. SNPs may be responsible for determining metabolic phenotype – ultra-rapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs), and poor metabolizers (PMs) – and the binding affinity to CYPs, all while affecting the half-life, as well as the therapeutic and detrimental effects of methadone.

3.1. CYP1 family

The CYP1 family consists of two subfamilies, encompassing three functional genes, *CYP1A1, 1A2*, and *1B1*. CYP1A1 and CYP1B1 enzymes are mainly expressed extra-hepatically, whereas CYP1A2 accounts for 4–16% of the hepatic CYPs and is responsible for 8–10% of the drugs metabolized by CYP isoforms, including methadone [41,54,58].

The interindividual variations in the mRNA and protein expression levels of CYP1A2 range from 15 to 40 fold, and a 40–130 fold inter-individual difference in activity [59]. To date, there are 190 genetic variants in *CYP1A2* [60,61]. An *in silico* study, by Wang et al. [52], found 31 nonsynonymous SNPs, of which 24 were predicted to be deleterious by the SIFT and PolyPhen algorithms. (S)-Methadone is marginally metabolized by CYP1A2 [46,62]. However, results of an *in vitro* study, conducted by Wang and DeVane [63], show neither the depletion of (R)- and (S)-methadone, nor the formation of EDDP by recombinant CYP1A2. Genetic variations in *CYP1A2* did not affect or had little influence on the plasma concentrations of methadone and EDDP [22,46,59]. Additionally, Crettol et al. [64]

observed *CYP1A2*1F* did not influence (R)-, (S)-, or (R,S)-methadone at the peak or trough levels in 245 MMT patients (96% white) (Table 1). Based on the current studies, *CYP1A2* SNPs have not been shown to impact the metabolism of methadone.

3.2. CYP2 family

The CYP2 family represents about 30% of human CYP isoforms, making it the largest CYP family [65,66]. The liver is comprised of five drug metabolizing CYP2 subfamilies (A–E), of which, the most pharmacologically important genes display the highest levels of polymorphism [54,55]. Methadone is metabolized by several members of the CYP2 family: 2B6, 2C8, 2C9, 2C18, 2C19, and 2D6.

3.2.1.   CYP2B—CYP2B6 has a 1–10% contribution to the total hepatic CYP pool, accounting for the metabolism of about 7–8% of pharmaceutical drugs [54,59]. Despite this enzymes' relatively low involvement in drug metabolism, CYP2B6 is the predominant determinant involved in the N-demethylation of methadone and clearance. CYP2B6 also displays stereoselectivity towards (S)-methadone [46,67]. *CYP2B6* is one of the most polymorphic genes, having 70 allelic variants and 38 protein variants, which affect catalytic activity, transcriptional regulation, and splicing. The variants also affect mRNA and protein expression, exhibiting nearly 300 fold interindividual variability [54,60,68–71].

CYP2B6 SNPs can alter the metabolic ratios of [methadone]/[EDDP]. A significant decrease in the metabolic ratio was observed in individuals carrying the *CYP2B6*2* allele, suggesting a role in an increased rate of metabolism. The data should be interpreted with caution because the subjects heterozygous for the variant only had a sample size of two [72]. A bioinformatics study also predicted *CYP2B6*2* to alter the metabolic phenotype as determined by both the SIFT and PolyPhen algorithms [52].

Lee et al. [73] studied the influence of *CYP2B6*4* on methadone plasma concentrations in 178 MMT patients in Taiwan. The study indicated an increase in (R,S)-methadone plasma levels in individuals homozygous for *CYP2B6*4.* The results were consistent with the Levran et al. [74] study of 74 Israeli MMT patients; though neither study showed any significant differences in the increase in (R,S)-methadone plasma levels. On the other hand, Kharasch et al. [75] observed *CYP2B6*4* carriers had a significant decrease in (R)-, (S)-, and (R,S)-methadone plasma levels, with increased methadone metabolism and clearance. It is important to note that one of the limitations to this study was the small sample size (n = 4). Gadel and colleagues [76] demonstrated an increase in N-demethylation of methadone by *CYP2B6*4* in an *in vitro* study, supporting the findings of Kharasch et al. [75].

CYP2B6*5 shows contradicting data in its involvement in methadone clearance and plasma concentrations. In a genotyping study of methadone-only overdoses in 125 Caucasians, *CYP2B6*5* was linked to a significant increase in (R,S)-methadone plasma levels [72]. In a study cohort consisting of 35 individuals with low (S)-methadone levels, *CYP2B6*5* was over-represented, indicating an increased CYP2B6 activity [32]. The methadone plasma concentration of two individuals genotyped for homozygosity in *CYP2B6*5* appeared to remain unaltered when compared to that of the wild type *CYP2B6*1* [75].

CYP2B6*6 is a haplotype consisting of two nonsynonymous variants, *CYP2B6*4* and *CYP2B6*9*. Numerous papers indicate an increase in (R,S)- and (S)-methadone plasma levels in *CYP2B6*6/*6* individuals [64,67,75,77,78]. Gadel et al. [79] and Kharasch et al. [75] have also demonstrated a decrease in methadone N-demethylation and decreased methadone clearance, respectively. An *in vitro* study found SNPs *CYP2B6*6* and *CYP2B6*9* to be catalytically deficient in EDDP formation [76]. Furthermore, increased (R,S)-methadone plasma levels were observed in 74 Israeli MMT patients homozygous for the *CYP2B6*9* SNP, though the increase was not statistically significant [74]. A significant increase in plasma concentrations and a decrease in (S)-methadone metabolism was observed in 366 Taiwanese MMT patients encompassing the *CYP2B6*9 SNP* [80]. The studies observing the SNPs of the haplotype *CYP2B6*6* suggest that the diminishing metabolic activity of CYP2B6*6/*6 is credited to the c.516G > T variant of *CYP2B6*9*.

Dobrinas et al. [32] observed an overrepresentation of *CYP2B6*11* polymorphism in a high (S)-methadone level group of MMT patients, indicating a decline in CYP2B6 activity. Wang et al. [80] examined polymorphisms in the intronic and 3′-untranslated region (UTR) regions of *CYP2B6*, and demonstrated the SNPs rs707265, rs2279345, rs1038376, and rs10403955 all correspond to increased [(S)-methadone/methadone dose] plasma ratios, and decreased (S)-methadone clearance (Table 1). The SNPs investigated in this section, demonstrate a compelling link to the importance of genetic variations in CYP2B6 and altered metabolism of methadone.

3.2.2. CYP2C—The CYP2C subfamily consists of four genes, *CYP2C8, 2C9, 2C18*, and *2C19*. The CYPs from these genes all contribute to the metabolism of methadone [46,77,81,82]. CYP2C9 is the highest expressed isozyme of the four in liver, while CYP2C8 and CYP2C19 are expressed at 2 and 10 fold lower levels than CYP2C9 [54]. CYP2C18 is expressed primarily in the skin [59]. CYP2C19 comprises 16% of the CYP2C family and metabolizes methadone to a greater extent, as compared to the CYP2C8, 2C9, and 2C18 isozymes [43]. The CYP2C family demonstrates stereoselectivity in methadone metabolism, where CYP2C8 and 2C19 predominately metabolize (R)-methadone, while CYP2C18 primarily metabolizes (S)-methadone.

Currently, there are no polymorphic studies on *CYP2C8* and *2C18* and their role in methadone metabolism. This is not surprising for *CYP2C18*, where only 8 variants have been reported to date [60,83]. There are 97 genetic variants observed in *CYP2C8* [60,84], which has a minor role in the metabolism of methadone, probably contributing to the dearth of studies in the corresponding SNPs.

Although *CYP2C9* exhibits 298 genetic variants and *2C19* has 111 reported genetic variants [60,85], only **2* and **3* have been examined for their respective CYP isozymes. Results from three separate studies showed no effect on methadone plasma levels with *CYP2C9*2* and *CYP2C9*3* genotypes in Caucasians [64,77,82]. A significant increase in methadone serum concentrations/dose ratio with heterozygous carriers of *CYP2C9*2* and *CYP2C9*3* SNPs was observed in a study in Norway. Similar results were seen for the homozygous *CYP2C9*2* and *CYP2C9*3* individuals, albeit, there was no significant differences in methadone serum concentrations/dose ratios [78].

In studies by Crettol et al. [64,77], *CYP2C19*2* and *CYP2C19*3* were also found to lack an effect on methadone plasma levels. A study of 366 Taiwanese MMT patients showed homozygous carriers of *CYP2C19*2* were associated with a significant increase in (R)-methadone levels. Patients homozygous for *CYP2C19*3* also tended to exhibit an increase in (R)-methadone levels, although the data was not significantly different [86]. Carlquist et al. [87] observed an increase in the plasma levels of EDDP with *CYP2C19*2* in 25 MMT patients (20 Caucasian, 4 Hispanic, and 1 African American), indicating an increase in methadone metabolism. Kringen et al. [78] noticed, in a Norwegian population, individuals homozygous and heterozygous carriers for *CYP2C19*2* and *CYP2C19*3* had an increase in methadone serum concentration/dose ratio, with significant differences seen only in heterozygotes. The contradicting results in the *CYP2C19* variants between these studies could be attributed to the ethnicity of the patients, where the frequencies of both *CYP2C19*2* and *CYP2C19*3* are higher in Asians than Caucasians (Table 1).

3.2.3.   CYP2D—The CYP2D subfamily consists of only one protein coding gene, *CYP2D6*, which accounts for 2–5% of the hepatic CYP composition and ~25% of pharmacological drug metabolism [54,88]. CYP2D6 metabolizes both enantiomers of methadone, with a slightly greater propensity towards (S)-methadone [46,63]. Unlike SNPs in other CYP genes, CYP2D6 phenotype is determined by allele combinations. PMs consist of two non-functional alleles, IMs are comprised of two decreased activity alleles, EMs have at least one functional allele, and UMs encompass more than one functional allele and/or an allele with a promoter mutation [82]. The major and most common mutation alleles are *CYP2D6*3, *4, *5*, and *6*, accounting for 93–97% of alleles among PM phenotypes [89,90]. *CYP2D6*1* and *CYP2D6*2* alleles represent normal functional activity.

In a 2001 study conducted by Eap et al. [89], CYP2D6 phenotypes had a significant influence on the (R)-, (S)-, and (R,S)-methadone blood concentrations, where higher concentrations were observed in PMs *(CYP2D6*4/*4, *4/*3, *4/*6)* and lower concentrations were measured in UMs *(CYP2D6*1/*1)*. Similarly, Crettol and colleagues [64], observed a 0.5 fold decrease in (S)-methadone and 0.7 fold decrease in (R)-methadone plasma levels in UMs *(CYP2D6*1/*1xN)* when compared to the EM/IM *(CYP2D6*1/*3, *1/*4, *1/*5, *1/*6)* group. In another study, the PM, IM, and EM phenotypes, representing alleles *CYP2D6*1, *1xN, *2, *3, *4, *4xN, *5, *6, *9, *10, *16, *28, *33*, and *41*, did not impact the clearance of (R)-, (S)-, or (R,S)-methadone [91]. *CYP2D6*3, *4, *5, *6, *7*, and *8* are non-functional variants, and *CYP2D6*9, *10*, and *41* have a decrease in function [91]. Fonseca et al. [82] observed EM patients received significantly lower doses of methadone as compared to UMs. It was also concluded that, contradictory to the phenotypes, UM *(CYP2D6*1xN, *2xN)* patients had significantly higher methadone plasma levels than EM *(CYP2D6*1, *2, *3, *6, *35)* and PM *(CYP2D6*4/*4)* patients. Further investigations on the *CYP2D6* alleles need to be conducted to get a clearer understanding of their significance. The relationship between *CYP2D6* allelic variants and phenotypic expression is depicted in Table 2.

3.3.   CYP3 family

The CYP3 family consists of only one subfamily, CYP3A, and four functional genes, *CYP3A4, 3A5, 3A7*, and *3A43*. These enzymes play a dominant role in drug metabolism pathways (~50%). CYP3A4 is the most expressed enzyme in adult liver, while CYP3A7 is predominantly expressed in fetal liver and is down-regulated after birth. CYP3A5 is polymorphically expressed in the liver, where alternative splicing results in multiple transcript variants, exhibiting high and low level proteins [54,88,92].

Methadone has been shown to be N-demethylated by CYP3A4, 3A5, and 3A7 in a non-stereoselective manner [46,93]. *CYP3A4* and *CYP3A5* have relatively low numbers of genetic variants, suggesting minor clinical importance in the clearance of their respective substrates [59,74]. This observation would be in line with the lack of polymorphic studies found for CYP3A and their role in methadone metabolism. However, in one study, the allelic frequency of *CYP3A4*1B* in 245 MMT patients (96% white) was observed and correlated with an increase in methadone plasma levels [64]. In the same study, the study cohort was genotyped for the deficient allele *CYP3A5*3*. The plasma levels of methadone were not influenced by the *CYP3A5* genotype. It is also interesting to note that there was a strong linkage between *CYP3A4*1B* and *CYP3A5*3* genotypes [64]. Similar results were observed by Fonseca et al. [82], where the genetic polymorphism of *CYP3A5* did not influence methadone plasma levels. One case report of a 25-year old male in a MMT program showed extensive metabolism of methadone to EDDP. The subject was found to be heterozygous for *CYP3A5*1 (CYP3A5*1/*3)* [94]. A large amount of CYP3A5 is expressed in individuals carrying at least one *CYP3A5*1* allele [94]. In a recent study of 155 serum samples and 62 patients, homozygous carriers of *CYP3A5*3* demonstrated a significant increase in methadone serum concentration/dose ratio, indicating a decrease in methadone clearance [78] (Table 1).

4.   Consequences of altered methadone metabolism

Polymorphisms in genes encoding CYP enzymes have the potential to shift their metabolic capacity or change the substrate specificity of the enzyme, eliciting variable consequences in drug treatment [95,96]. These changes can be crucial in the therapeutic potency of methadone. The main metabolic pathway of methadone involves the N-demethylation by hepatic CYPs into the primary metabolite, EDDP. Changes in the rate of EDDP formation could influence the pharmacodynamics of methadone.

CYP2B6*4 was the only SNP that was found to increase the clearance of methadone by the N-demethylation of both (R)- and (S)-methadone [75,76]. In turn, there would be a decrease in the disposition of (R,S)-methadone, reducing the pharmacological effect of methadone and inducing withdrawal symptoms. Individuals with this SNP may require a higher dose of methadone.

Several SNPs were found to increase the methadone plasma concentration due to a decrease in the metabolic activity of the CYPs. Three independent studies collectively demonstrated elevated plasma levels of (R,S)- and (S)-methadone with diminishing N-demethylation of (R)- and (S)-methadone due to *CYP2B6*2, *9* and **11* [32,72,74,76,80]. *CYP2B6*6* is a

haplotype consisting of both *CYP2B6*4* and *CYP2B6*9* variants. Carriers of *CYP2B6*6/*6* had diminished methadone metabolism and clearance, hence, an increase in methadone levels. This activity is credited to the *CYP2B6*9* SNP, where individuals with *CYP2B6*4/*6* haplotype did not observe the metabolic inhibition [64,67,75–79]. Mutations located in the UTR and intronic regions are also relevant in the biotransformation of methadone. Table 1 shows the four SNPs in the UTR and intronic regions linked to a decreased clearance and increased concentration of methadone [80]. CYP2B6 influences the metabolism of (S)-methadone and, to a far lesser extent,(R)-methadone. Individuals with the genotypes mentioned above, particularly *CYP2B6*6/*6*, have a greater risk of suffering torsades de pointes and sudden death. A lower dose of methadone may be required for treatment for individuals with these variants.

Reduced metabolism by CYP2C19 can lead to increased plasma concentrations of methadone, specifically (R)-methadone, which can potentiate depressed ventilation manifesting as a potentially fatal respiratory depression. Both *CYP2C19*2* and *CYP2C19*3* were associated with elevated methadone plasma concentrations [78,86]. However, these results could be variable in different ethnic groups.

CYP2D6 and CYP3A4/5 metabolize both enantiomers of methadone, though CYP2D6 has a slightly greater preference towards (S)-methadone. Decreased metabolism by these CYPs may heighten either detrimental effect due to augmented levels of methadone. *CYP3A4*1B* has been shown to increase (S)-methadone plasma levels [64], which can lead to cardiotoxic effects. In a study of 136 Caucasian individuals who died of methadone-only overdosing, an enrichment was observed in the *CYP3A4*1B SNP* [97]. *CYP3A5*3* is a common SNP with high frequency in the Caucasian population, where a splicing defect causes a loss of function of the *CYP3A5* enzyme [58]. This is exemplified in a couple of studies where the methadone plasma levels are elevated in *CYP3A5*3/*3* individuals, while metabolism is increased and plasma levels are decreased in individuals carrying at least one copy of *CYP3A5*1* [79,94]. The effects of SNPs located on the *CYP2D6* gene is slightly more complicated. CYP2D6-dependent metabolism of methadone has been studied based on the phenotypic variability determined by a combination of SNPs on the *CYP2D6* gene. There have not been any clear implications on the relationship between *CYP2D6* SNPs and methadone metabolism and their role on the pharmacokinetics and pharmacodynamics of methadone.

Since methadone is metabolized by a number of CYPs, a combination of any of the SNPs, within or between genes, can have varying effects on the pharmacological response of methadone treatment. A genome-wide pharmacogenomic study showed two *CYP2B6* haplotypes of rs8100458, rs7250601, rs7250991, rs11882424, rs8192719, and rs10853744 (T-A-A-T-C-G and T-C-C-T-T-T) accounted for the variation in (S)-methadone plasma levels [98]. In order to understand the complexity of the effects of CYP SNPs on methadone pharmacokinetics and pharmacodynamics, inclusion of additional gene variants and haplo-type variants is required. Though SNPs influence only part of methadone pharmacology, the impact of genetic variations can be compounded by drug interactions, environmental factors, sex, health, and other nongenetic factors. Co-administration of methadone with respiratory depressants, such as other opioids, alcohol, or benzodiaze-pines, may lead to a greater

detrimental effect. Likewise, individuals with reduced liver function (e.g. cirrhosis) could have a reduced capacity to metabolize methadone.

5. Conclusion

Methadone is currently used as a treatment and maintenance therapy for opioid addiction and as an analgesic for severe chronic pain. The wide-ranging half-life of methadone may result in some individuals having higher than normal therapeutic levels leading to negative, detrimental, and fatal side effects. Elevated (R)-methadone levels may lead to respiratory depression, while an increase in plasma levels of (S)-methadone may lead to severe cardiac arrhythmias. A significant portion of opioid-related deaths have been contributed to the use of prescription methadone for pain management. The pharmacogenetics of an individual possesses the ability to affect the pharmacokinetics and pharmacodynamics of methadone. SNPs located on CYP genes shift the metabolic capacity or change the substrate specificity of the CYP enzyme, affecting the metabolism of methadone. SNPs *CYP2B6*6, *9, *11, CYP2C19*2, *3, CYP3A4*1B*, and *CYP3A5*3* result in increased methadone plasma concentrations, decreased N-demethylation, and decreased methadone clearance. Since CYP2B6 is the major determinant of methadone clearance, *CYP2B6*6/*6* is of particular interest. Homozygous carriers of *CYP2B6*6/*6* expressed diminished methadone metabolism and clearance, thus these individuals have a greater propensity for detrimental adverse effects. However, *CYP2B6*4* demonstrated an increase in N-demethylation and methadone clearance. A better understanding of the role of CYP SNPs in methadone metabolism can improve the proper therapeutic dosing for methadone, patient outcome, and the development of individualized medicine.

Abbreviations:

ADH	alcohol dehydrogenase
CDC	Centers for Disease Control and Prevention
CYP	cytochrome P450
Del	deletion
EDDP	2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine
EM	extensive metabolizer
EMDP	2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline
FDA	Food and Drug Administration
hERG	human ether-a-go-go related gene
IM	intermediate metabolizer
MMT	methadone maintenance treatment
MOR	μ-opioid receptor

MTD	methadone
NCBI	National Center for Biotechnology Information
p-HO	para-hydroxy
PM	poor metabolizer
SNP	single nucleotide polymorphism
UM	ultra-rapid metabolizer
UTR	untranslated region

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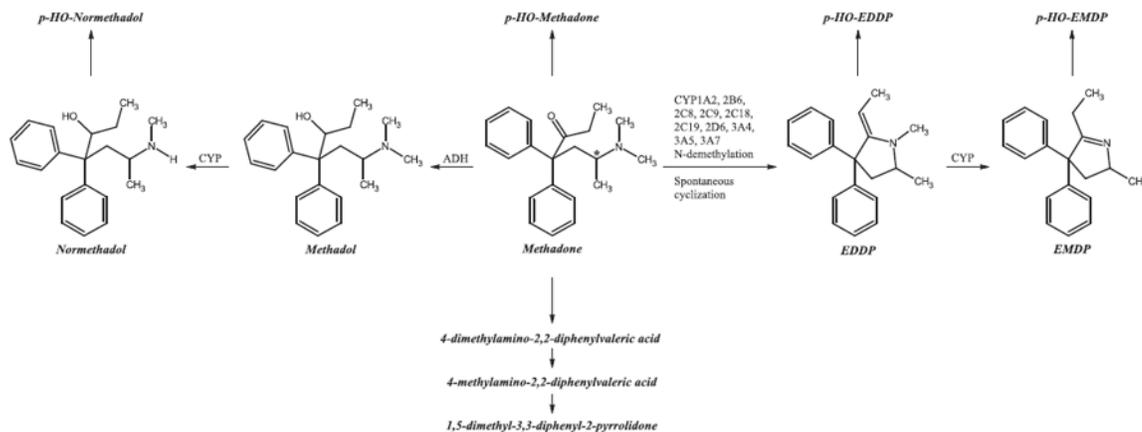
Fig. 1.
Eleven methadone metabolites found in human excretion. *Indicates chiral carbon atom. ADH, alcohol dehydrogenase; CYP, cytochrome P450; EDDP, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine; EMDP, 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline; p-HO, para-hydroxy.

Table 1

Cytochrome P450 single nucleotide polymorphisms relevant to methadone metabolism.

Alleles	dbSNP ID[†]	Key sequence change[‡]	Effect and key amino acid substitution (if any)	Effect on enzyme activity	Ref.
*CYP1A2*1F*	rs762551	intron 1, c.–9–154C > A		No influence on (R)-, (S)-, or (R,S)-MTD levels at trough or peak	[64]
*CYP2B6*2*	rs8192709	c.64C > T	R22C	↑ [(R,S)-MTD]/[EDDP] ratio	[72]
*CYP2B6*4*	rs2279343	c.785A > G	K262R	↑ (R,S)-MTD plasma levels	[73,74]
				↓ (R,S)-MTD plasma levels	[75,76]
				↑ MTD clearance	
				↑ (R)- and (S)-MTD N-demethylation	
*CYP2B6*5*	rs3211371	c.1459C > T	R487C	↓ (S)-MTD plasma levels	[32]
				↑ (S)-MTD clearance	
				No effect on MTD clearance	[75]
				↑ (R,S)-MTD plasma levels	[72]
*CYP2B6*6*	rs2279343	c.785A > G	K262R	↑ (S)- and (R,S)-MTD plasma levels	[64,67,75–79]
	rs3745274	c.516G > T	Q172H	↑ MTD/Dose plasma ratio	
				↓ (R)- and (S)-MTD N-demethylation	
				↓ MTD clearance	
*CYP2B6*9*	rs3745274	c.516G > T	Q172H	↑ (R,S)-MTD plasma levels	[74,76,80]
				↑ [(S)-MTD/MTD Dose] plasma ratio	
				↓ (R)- and (S)-MTD N-demethylation	
				↓ (S)-MTD clearance	
*CYP2B6*11*	rs35303484	c.136A > G	M46V	↑ (S)-MTD plasma levels	[32]
				↓ (S)-MTD clearance	
CYP2B6 3′ UTR	rs707265	c.*1355A > G		↑ [(S)-MTD/MTD Dose] plasma ratio	[80]
				↓ (S)-MTD clearance	
CYP2B6 3′ UTR	rslO38376	3'UTR, c.*1277A > T		↑ [(S)-MTD/MTD Dose] plasma ratio	[80]
				↓ (S)-MTD clearance	
CYP2B6 intron 1	rsl0403955	c.172–468 T > G		↑ [(S)-MTD/MTD Dose] plasma ratio	[80]
				↓ (S)-MTD clearance	
CYP2B6 intron 5	rs2279345	c.923–197T > C		↑ [(S)-MTD/MTD Dose] plasma ratio	[80]
				↓ (S)-MTD clearance	
*CYP2C9*2*	rsl799853	c.430C > T	R144C	No effect on MTD plasma levels	[64,77,82]
				↑ MTD/Dose plasma ratio	[78]
*CYP2C9*3*	rsl057910	c.1075A > C	I359L	No effect on MTD plasma levels	[64,77,82]

Alleles	dbSNP ID[†]	Key sequence change[‡]	Effect and key amino acid substitution (if any)	Effect on enzyme activity	Ref.
				↑ MTD/Dose plasma ratio	[78]
CYP2C19*2	rs4244285	c.681G > A	I331V; splicing defect	No effect on MTD plasma levels	[64,77,82]
				↑ (R)-MTD plasma levels	[78,86]
				↑ MTD/Dose plasma ratio	
				↑ EDDP plasma levels	[87]
CYP2C19*3	rs4986893	c.636G > A	W212X; stop codon	No effect on MTD plasma levels	[64,77]
				↑ MTD/Dose plasma ratio	[78]
CYP3A4*1B	rs2740574	5'UTR, C, −392A > G		↑ (S)-MTD plasma levels	[64]
CYP3A5*3	rs776746	5'UTR, c.219–237A > G	Splicing defect	No effect on MTD plasma levels	[64,82]
				↑ MTD metabolism	[94]
				(*1/*3 carrier)	
				↑ MTD/Dose plasma ratio (*3/*3)	[78]

More comprehensive information is available from the Human Cytochrome P450 Allele Nomenclature Committee [69].

CYP, cytochrome P450; MTD, methadone; EDDP, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine; UTR, untranslated region.

[†]National Center for Biotechnology Information (NCBI) dbSNP [88].

[‡]Location of sequence change on mRNA for the respective CYP NM accession number [88].

Table 2

Cytochrome P450 2D6 and their corresponding activity and phenotype.

Alleles	dbSNP ID[†]	Key sequence change[‡]	Effect and key amino acid substitution (if any)	Effect on enzyme activity	Phenotype	Ref.
CYP2D6*1				Wild type Duplication of gene	EM	[57,64,82,89,91]
CYP2D6*lxN				Multiduplication of gene	UM	[64,82,91]
				N active genes		
				↑ activity		
CYP2D6*2	rsl6947	c.886C > T	R296C	Normal activity	EM	[57,82,89,91]
	rsl 135840	c.1457G > C	S486T	Duplication of gene		
CYP2D6*2xN				Multiduplication of gene	UM	[82]
				N active genes		
				↑ activity		
CYP2D6*3	rs3574686	c.775delA	259Frameshift	Defective allele	PM	[64,82,89,91]
CYP2D6*4	rsl 065852	c.lOOC > T	P34S	Defective allele	PM	[57,64,82,89,91]
	rs28371703	c.271C > A	L91M			
	rs28371704	c.281A > G	H94R			
	rs3892097	c.506–1G > A	Splicing defect			
	rsl 135840	c.1457G > C	S486T			
CYP2D6*4xN				Defective allele Multiduplication of gene	PM	[91]
CYP2D6*5				Deletion of entire CYP2D6 gene	PM	[57,64,91]
CYP2D6*6	rs5030655	c.454delT	118Frameshift	Defective allele	PM	[64,82,89,91]
CYP2D6*7	rs5030867	c.971A > C	H324P	Defective allele	PM	[91]
CYP2D6*8	rs5030865	c.505G > A	G169R	Defective allele	PM	[91]
	rsl 6947	c.886C > T	R296C			
	rsl 135840	c.1457G > C	S486T			
CYP2D6*9	rs5030656	c.841_843delAAG	K281del	Impaired function	IM	[91]
CYP2D6*10	rsl 065852	c.lOOC > T	P34C	Impaired function	IM	[57,91]
	rsl 135840	c.1457G > C	S486T			
CYP2D6*16		CYP2D7/2D6 hybrid	Frameshift; switch region exon7-intron 8		PM	[91]
CYP2D6H*17	rs28371706	c.320C > T	T107I	Impaired function	IM	[57]
	rsl 6947	c.886C > T	R296C			
	rsl 135840	c.1457G > C	S486T			
CYP2D6*28	rs78482768	19G > A	V7M	Unknown	Unknown	[91]
	rsl 6947	c.451C > G	Q151E			
	rsl 135840	c.886C > T	R296C			
		c.1457G > C	S486T			
CYP2D6*29	rs61736512	c.406G > A	V136I	Impaired function	IM	[57]
	rsl 6947	c.886C > T	R296C			

Alleles	dbSNP ID[†]	Key sequence change[‡]	Effect and key amino acid substitution (if any)	Effect on enzyme activity	Phenotype	Ref.
	rs59421388	c.1012G > A	V338M			
	rsl 135840	c.1457G > C	S486T			
CYP2D6∗33	rs28371717	c.709G > T	A237S	Normal activity	EM	[91]
CYP2D6∗35	rs769258	c.31G > A	V11M	Normal activity	EM	[57,82]
	rsl 6947	c.886C > T	R296C	Duplication of gene		
	rsl 135840	c.1457G > C	S486T			
CYP2D6∗36	rsl 065852	c.lOOC > T	P34S	Defective allele	PM	[57]
	rsl 135840	c.1457G > C	S486T			
CYP2D6∗41	rsl 6947	c.886C > T	R296C	Impaired function	IM	[91]
	rs28371725	c.985 + 39G > A	Splicing defect			
	rsl 135840	c.1457G > C	S486T			
CYP2D6∗43	rs28371696	c.77G > A	R26H	Normal activity	EM	[57]
				Duplication of gene		
CYP2D6∗45	rs28371710	c.463G > A	E155K	Normal activity	EM	[57]
	rsl 6947	c.886C > T	R296C	Duplication of gene		
	rsl 135840	c.1457G > C	S486T			

More comprehensive information is available from the Human Cytochrome P450 Allele Nomenclature Committee [69].

CYP, cytochrome P450; del, deletion; EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer; UM, ultra-rapid metabolizer.

[†]National Center for Biotechnology Information (NCBI) dbSNP [88].

[‡]Location of sequence change on mRNA for the respective CYP NM accession number [88].