Therapeutic Drug Monitoring to Support Clinical Pharmacogenomics

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Abstract The implementation of pharmacogenomics can improve the efficacy of therapeutic drugs while reducing the incidence of side effects and drug toxicity. Therapeutic drug monitoring is well accepted and widely practiced for many drugs and is also relevant for drugs for which pharmacogenomic testing is needed. Tamoxefin is metabolized by CYP 2D6 to endoxifen, clopidogrel by CYP 2C19 to thiol-containing active metabolite, and opioid drugs by 2D6 to morphine and other metabolites. For these drugs, genetic testing can be used to predict efficacy for breast cancer outcomes, freedom from cardiovascular events, and adequate pain control, respectively. Therapeutic drug monitoring (TDM) can be used to determine drug compliance, especially for the opioids which have street value and can be diverted as a drug of abuse. Drug levels can be used to titrate drug dosage for individuals who are shown to be sub-therapeutic. TDM can also improve efficacy for tamoxifen for patients taking drug inhibitors, and be useful for determining the mechanism of clopidogrel resistance (i.e., pharmacokinetics vs. pharmacodynamics). Since there are no specific immunoassays for these drugs and metabolites for serum measurements, liquid chromatography/mass spectrometric methods will be necessary to implement TDM.

1 Introduction

"Personalized medicine" is a new medical approach that attempts to personalize medical treatment to the specific needs of the patient. Regarding drug therapeutics, it is a move from "one size fits all" to the "right drug" at the "right dosage" to the

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Table 1 Relevant pharmacogenomic drugs and possible phenotypic assessments

Drug	Genotype target	Phenotype target
Warfarin	CYP 2C9 and VKORC1[a]	Prothrombin time/INR[b]
Azothioprine	TPMT[c]	Red cell TPMT
Tamoxifen	CYP 2D6	Endoxifen
Clopidogrel	CYP 2C19	R-130964 metabolite
		Platelet aggregometry
Codeine	CYP 2D6	Morphine

[a]Vitamin K reductase epoxide complex
[b]International normalized ratio
[c]Thiopurine methyltransferase

"right person" at the "right time." Pharmacogenomics is a science that predicts therapeutic efficacy and toxicity avoidance by tailoring drug therapy according to an individual's genetic makeup. Polymorphisms in the genes that encode the enzymes necessary in the inter- and intracompartmental transportation, metabolism, and excretion of therapeutic drugs can have a major effect on the performance of a drug in clinical practice. Currently, genotyping for the family of cytochrome (CYP) microsomal enzyme systems is important in identifying individuals as slow, intermediate and ultrarapid metabolizers relative to the wild type. There are specific cytochrome isoenzymes that are responsible for the majority of the drugs that are detoxified by the liver. Homozygous subjects who are slow metabolizers produce enzymes that are defective and have low enzyme activity. Homozygous intermediate metabolizers produce enzymes that have reduced function. Alternately, an intermediate metabolizer could result from a heterozygous individual who has one wild-type copy and one null gene. Ultrarapid metabolizers have more "gene duplications," i.e., more than the usual two copies of the CYP enzyme.

An individual's germ line "genotype" is created at birth and remains largely unchanged during life. The major advantage of genotyping is that a determination can be made in the absence of the drug itself. However, while the coded protein is defined by the genotype, an individual's "phenotype" is dependent on genetic and nongenetic factors. The level of RNA expression for an individual can be variable in the amount of protein produced, and greatly affect the individual's phenotype. It has been known for many years that the expression of the CYP enzymes from the liver can be enhanced or inhibited by the presence of other drugs. Thus, for clinical pharmacogenomics, measurement of the concentration of the drug or its metabolites may be a better reflection of its enzyme activity. A disadvantage of drug measurements is that for an accurate assessment of concentrations, the individual must be regularly taking the drug at the prescribed dosage, and be at pharmacologic steady state. During the drug induction phase, this might expose the individual to unwarranted and unexpected side effects for some medications. For drugs with long half-lives, there may be a significant delay in the assessment of efficacy by therapeutic drug monitoring (TDM) levels. Clearly, the effective therapeutic management of many therapeutic drugs today is a combination of genotypes with phenotypes. Table 1 lists some of the drugs that warrant pharmacogenomic testing, and the relevant phenotypic targets.

2 LC-MS/MS to Support Clinical Pharmacogenomics

2.1 Tamoxifen

2.1.1 Pharmacology and Pharmacogenetics

Tamoxifen is a widely available hormonal adjuvant therapy for women with breast cancer, and is most effective on tumors that are positive for estrogen and progesterone receptors. While alternative drugs are available, such as the aromatase inhibitors, tamoxifen remains the drug of choice for premenopausal breast cancer patients. Tamoxifen exists as a prodrug that must be converted to endoxifen, the metabolite that has about 100-fold higher activity in blocking estrogen receptors than the parent drug [1]. The conversion of tamoxifen to endoxifen occurs through two metabolic pathways. The major pathway is catalyzed principally by CYP 3A4/5 and first to *N*-desmethyltamoxifen which has minimal biologic activity, and then to endoxifen by CYP 2D6. The minor pathway is conversion by CYP 2D6 to 4-hydroxytamoxifen, which has significant biologic activity but is present in low blood concentrations, and then to endoxifen by CYP 3A4/5 [2]. The rate-limiting step in either pathway is the enzyme CYP 2D6. Individuals who have CYP 2D6 genotypes that encode a null enzyme (e.g., *2D6 *3* through **8*) have reduced concentrations of endoxifen relative to wild-type individuals. Those who have a reduce enzyme activity genotypes (e.g., *2D6 *9, *10, *17,* and **29*) also have lower endoxifen concentrations. Retrospective clinical trials involving tamoxifen have shown that patients who are poor (2D6 **4/*4*) or intermediate (2D6 **10/*10*) have significantly shorter time to breast cancer recurrence and worse relapse-free survival [3, 4]. Other researchers have shown that there is a trend towards reduced endoxifen concentrations, with the highest concentrations seen in ultra (2D6 gene duplication) and extensive metabolizers (**1/*1*), successively lower levels with one or more copies of the reduced activity genes (**10*), and lowest concentrations with one or more copies of the null genes (**10*). The hypothesis is that worsening clinical outcomes are associated with decreasing endoxifen concentrations. Madlensky et al. recently showed that when patients are divided into quintiles, there is a threshold effect, i.e., only in the lowest endoxifen quintile of values was associated with poor outcomes [5]. As such, there are no consequences of having an excess endoxifen concentration, such as expected for individuals who have more than two copies of the 2D6 wild-type gene and are ultrarapid drug metabolizers.

2.1.2 Nongenetic Factors

While genotyping is an important first step in predicting individuals who will not benefit from tamoxifen treatment, there are nongenetic factors that also result in lowering of tamoxifen concentrations. The most widely studied are the class of drugs that are serotonin selective reuptake inhibitors (SSRI) such as paroxetine and fluoxetine [6]. These drugs are used to treat depression, hot flashes, and vaginal dryness and are potent inhibitors of CYP2D6 enzyme activity. Borges et al. showed

that endoxifen concentrations among 2D6 wild-type patients who were 2D6 genotypes and were on SSRIs had endoxifen concentrations that were as low as poor metabolizers [7]. In a population-based study of 2,430 breast cancer patients treated with tamoxifen, those on a single SSRI had an increased risk of cancer deaths [8]. These and other investigators have strongly suggested the discontinuance of these drugs. Patients may not readily disclose their antidepressant drug use to their oncologists due to the stigma associated with depression. While the information for SSRIs is now well documented, there are many other potential 2D6 enzyme inhibitors that are taken by breast cancer patients. Herbal medications in particular are widely used and may have adverse effects on tamoxifen metabolism. While Wu et al. showed no difference with self-reported soy food intake, there are many other herbals that have not yet been tested [9]. In the absence of outcome evidence from clinical trials, there may be a role in routine therapeutic drug monitoring for the active metabolite. LC-MS measurement for the occult use of SSRI may also be warranted.

2.1.3 Analytical Assays and TDM Testing

LC/tandem MS assays for tamoxifen metabolites have been described by numerous investigators and used for research purposes [10–12]. Figure 1 shows a typical chromatogram for the analyses of these metabolites from a serum sample. Following chronic administration of a standard 20 mg tamoxifen per day dosage on presumably wild-type subjects, typical serum concentrations at steady state were 150 (±50) ng/mL for tamoxifen, 180 (±70) ng/mL for *N*-desmethytam, 2.5 (±1.2) ng/mL for 4-hydroxytam, and 5.0 (±2.5) ng/mL for endoxifen [10].

Assays for tamoxifen and its metabolites are currently not used for routine therapeutic drug monitoring. Because the half-life of tamoxifen is long, TDM for tamoxifen has the disadvantage over genotyping in that steady state concentrations are not reached for 2–3 months. A significant delay in prescribing the most effective adjuvant therapeutic regimen (i.e., tamoxifen vs. aromatase inhibitors) may have an impact on disease-free survival. Therefore, a combination of genotyping and phenotyping might be the best approach. Figure 2 shows a proposed algorithm combining the attributes of both strategies. This scheme has not been clinically validated nor is it endorsed by any clinical practice groups. An alternative use of TDM is to evaluate the effectiveness of increasing the tamoxifen dose from the standard 20 to 40 mg/day for individuals who are intermediate metabolizers. The National Cancer Institute is evaluating this protocol in a prospective trial [13].

2.2 Clopidogrel

2.2.1 Pharmacology and Pharmacogenomics

Clopidogrel is part of the thienopyridine class of antiplatelet drugs that are widely used to treat patients with cardiovascular disease. The American College of Cardiology has recommended antiplatelet medications for patients after angioplasty

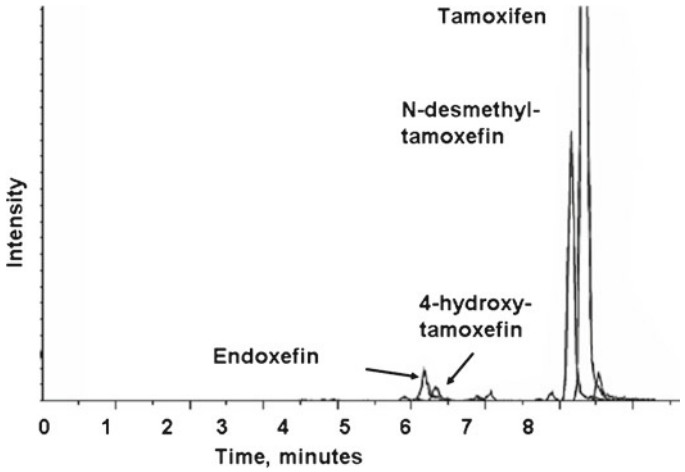

Fig. 1 Representative chromatograms of the LC/MS/MS analysis of tamoxifen and its principal metabolites from serum of a patient with breast cancer. EM, IM, PM, extensive, intermediate, and poor metabolizers, respectively, for 2D6. TDM, therapeutic drug monitoring. Tamoxifen 205 ng/mL, endoxifen 32.8 ng/mL, *N*-desmethyl tamoxefin 320 ng/mL, 4-ohydroxytamoxefin 5.6 ng/mL

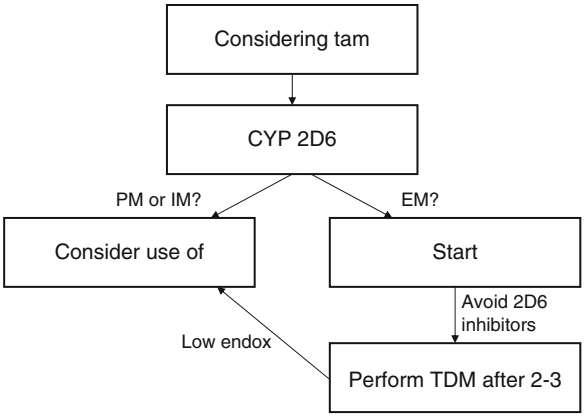

Fig. 2 Proposed testing algorithm for tamoxifen therapy in breast cancer

and stent placement [14]. The drug binds to the $P2Y_{12}$ platelet receptor thereby blocking the actions of agonists such as adenosine diphosphate (ADP). Like tamoxifen, clopidogrel is a prodrug that must be converted to an active metabolite for full pharmacologic action. Clopidogrel is first metabolized to 2-oxoclopidogrel by CYP 1A2, 2C19, and 2B6 and then to the R-130964 metabolite through CYP 2A, 2C9, 2C19, and 2B6 [15]. Of these enzymes, only the loss of function variants in CYP 2C19 and CYP 2C9 affect the pharmacokinetics to any appreciable extent. The effect for 2C19 polymorphism is greater than for 2C9 alone as individuals with a heterozygous **1/*2* genotype had no effect on the drug's kinetics [15] (Fig. 3).

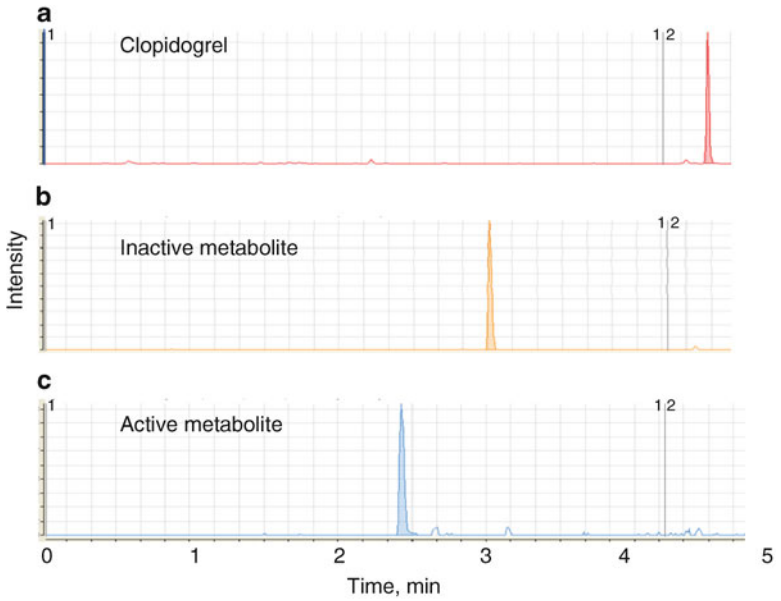

Fig. 3 Representative ion chromatograms for the LC/TOF MS analysis of (**a**) clopidogrel, (**b**) inactive metabolite, and (**c**) active metabolite. The latter is derivatized with 2-bromo-3′-methoxyacetophenone. Separate injections were necessary for the active vs. inactive metabolite due to the need to derivatize the biologically active clopidogrel form

There have been several clinical studies on the effect of CYP 2C19 carriers of at least one loss of function alleles. In the TRITON-TIMI39 study, carriers had a relative increase of 53 % in the incidence of death from cardiovascular disease, myocardial infarction, or stroke compared to noncarriers and a threefold higher risk of stent thrombosis [16]. In the Collet study among young patients, the hazard ratios (HR) were 3.69 and 6.02 ($p<0.05$) for cardiac events and stent thrombosis, respectively [17]. Similar findings were also reported by Giusti et al. (HR = 2.36 and 2.59 for mortality and stent thrombosis, respectively, $p<0.05$) [18]. Based on these and other reports, the US Food and Drug Administration (FDA) issued a Black Box warning in March 2010 on the use of clopidogrel, addressing the need for pharmacogenomic testing [19]. While it was appropriate for the FDA to issue this warning, there is insufficient evidence to date to recommend the actions taken (e.g., higher clopidogrel dosing or use of an alternative medication) for individuals who are determined to be at risk [20]. Such guidance will likely follow with completion of ongoing randomized trials addressing these issues. GRAVITAS was a randomized clinical trial of 75 mg vs. 150 mg clopidogrel for individuals who are resistant to clopidogrel by platelet aggregometry, with 1 and 6-month outcomes recorded [21].

Recently, the CYP 2C19 **17* variant was identified as having increased transcriptional activity, resulting in ultrarapid metabolism. For clopidogrel, the consequence is

an increased concentration of the active metabolite. Sibbing et al. showed that individuals with the **17* polymorphism were associated with an increased risk of bleeding [22]. Although not specifically mentioned in the FDA Black Box warning, testing for this variant is also indicated. Clinicians should be cautioned by clinical laboratorians who only test for the CYP **2* and **3* polymorphism, as the wild type (**1*) will be erroneously inferred in the absence of direct testing for the **17* variant.

2.2.2 Nongenetic Factors and Pharmacodynamics

Individuals with genetic variances to hepatic enzymes such as CYP 2C19 represent a pharmacokinetic mechanism towards drug resistance, i.e., insufficient concentrations of the active metabolite. These are a subset of individuals who have clopidogrel resistant as measured by functional testing. There are several methods to measure platelet function as an assessment of the pharmacodynamics of clopidogrel, including as light transmittance aggregometry, impedance measurement of whole blood aggregometry, vasodilator-stimulated phosphoprotein analysis (VASP) as measured by flow cytometric analysis, and various commercial assays and platforms such as the PFA100 (Siemens Healthcare, Deerfield, IL), VerifyNow (Accumetrics, Carlsbad, CA), and Impact-R (Cresier, Switzerland) [23]. Individuals can be resistant to clopidogrel even if they are wild type for CYP 2C19. The mechanisms include polymorphism in other metabolic enzymes such as CYP 2C9, drug–drug interactions, variable drug absorption or clearance, P2Y12 receptor variability such as an increase in the number of receptors or upregulation of alternate platelet activation pathways [24]. A combination of testing for the pharmacokinetic (CYP 2C19) and pharmacodynamic (platelet function testing) provides the most insight for a particular patient.

2.2.3 Analytical Assays and TDM Testing

The absence of a specific therapeutic algorithm based on pharmacogenomic and platelet function testing that has been endorsed by international cardiology societies has slowed the adoption of testing into routine clinical practice. An important parameter that would add to the understanding of clopidogrel resistance in a particular patient would be therapeutic drug monitoring for the active metabolite. While LC tandem MS assays have been described for the carboxylic acid metabolite [25, 26], this product is inactive and will not likely be clinically useful. More recently, an LC-MS assay has been developed for the active metabolite [27]. Accurate analysis requires production of the thiol group with alkylating agents such as *N*-ethylmaleimide added to the blood sample within a few minutes of collection.

Measurement of the concentration of the active metabolite can be used to assess clopidogrel efficacy for patients who have a pharmacokinetic mechanism for platelet resistance. Mega et al. showed that patients who are intermediate or slow metabolizers

for CYP 2C19 have lower concentrations of the active metabolite than wild-type patients [16]. By monitoring the active metabolite concentration, a physician may be able to titrate the clopidogrel dosage for intermediate and poor metabolizers to match the levels seen in noncarriers. Assuming that these individuals do not also have a pharmacodynamic reason for their platelet resistance, increasing the drug dosage should reduce the rate of adverse cardiac events to that of wild types. A loading dose titration study was previously conducted by Bonello et al. using VASP testing as the therapeutic monitoring indicator [28]. Major adverse event rates were significantly lower in the VASP-guided group than controls with no increase in the incidence of major or minor bleeding. However, as much as 2,400 mg of clopidogrel was needed given, some eightfold higher than the recommended bolus dose of 300 mg. While this study was successful, many physicians will likely be hesitant to use this high dosage. In patients who are resistant to clopidogrel by platelet function tests and are wild type for CYP 2C19, a consideration can be made to switch to an alternate antiplatelet medication such as prasugrel, which is not affected by CYP 2C19 [15]. Figure 4 illustrates a proposed algorithm based on genotyping, functional testing, and phenotyping through therapeutic drug monitoring measurements. This scheme has not been clinically validated, nor is it endorsed by any clinical practice groups.

2.3 Opioids for Pain Management

2.3.1 Pharmacology and Pharmacogenomics

Opioid analgesics are used to treat moderate to severe pain. They function by decreasing pain perception and increasing pain tolerance. Opioids are highly prescribed; however, there is a large degree of variability in individual responses to opioids. The majority of opioids used in pain management are metabolized by CYP450 enzymes. *CYP2D6* is the primary enzyme responsible for the formation of the active metabolites of codeine, hydrocodone, dihydrocodeine, oxycodone, and tramadol. *CYP2D6* is the only noninducible CYP450 enzyme, thus genetic variation is the main source for interindividual differences in enzyme activity. *CYP2D6* is highly polymorphic. Over 90 distinct allelic variants have been identified [29]. These include single nucleotide polymorphisms, haplotype, and copy number variants. These variants result in a large degree of metabolic and phenotypic diversity within populations. *CYP2D6* variants can be categorized into ultrarapid metabolizers (UM), extensive (EM), intermediate (IM), and poor metabolizers (PM). An individual's highest functioning *CYP2D6* allele predicts his/her phenotypic activity. EMs are considered phenotypically normal and have at least one functional *CYP2D6* allele. UMs have multiple gene copy variants and may experience toxicity for opioids (i.e., codeine) due to increased levels of their active metabolites (i.e., morphine). IMs and PMs have a decreased ability to metabolize *CYP2D6* substrates compared to EMs, and may be at risk for adverse effects from higher plasma levels

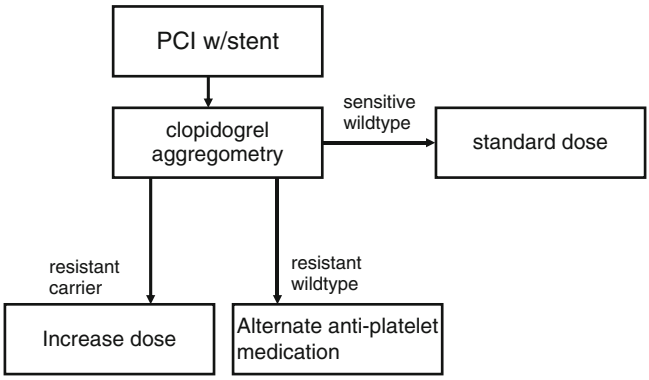

Fig. 4 Proposed testing algorithm for clopidogrel therapy for acute coronary syndromes

of the parent drug. However, IMs and PMs may experience a lack of efficacy from some opioids (i.e., codeine) because of their inability to form the active metabolite (i.e., morphine). The majority of individuals are *CYP2D6* EMs; however, 7–10 % of the Caucasian population and 1–4 % of other ethnic populations have nonfunctional alleles [29, 30].

CYP2D6 is the most well studied gene with respect to the pharmacogenetics of codeine metabolism. Approximately 50–70 % of codeine is glucuronidated to codeine-6-glucuronide by UGT2B7 and 10–15 % is *N*-demethylated to norcodeine by CYP3A4 [31]. Compared to codeine both metabolites have a similar affinity for the μ-opioid receptor. A smaller percentage of codeine (0–15 %) is *O*-demethylated to morphine which has a 200-fold increased affinity for the μ-opioid receptor compared to codeine. PMs may not experience adequate pain relief since they are unable to convert codeine to morphine, while UMs may experience morphine intoxication as a result of rapid conversion of codeine to morphine.

Multiple pharmacogenetic studies have shown that there is significant variability in both the pharmacokinetics and pharmacodynamics of codeine and that its analgesic effects are mostly dependent on metabolism to morphine [32–35]. However, many of these studies were small and had a limited sample-size. Large-scale studies are still needed to demonstrate impaired analgesic outcome in *CYP2D6* PMs. In 2002, Williams and colleagues investigated the postoperative analgesic efficacy in a pediatric population ($n=46$) by determining genotype, phenotype and morphine production from codeine [32]. They found that there was a significant relationship between phenotype and plasma morphine concentration after administration of codeine, however, no relationship was found between phenotype and analgesia. This could be a result of experimental cofounders such as coadministration with diclofenac. Another study found that *CYP2D6* UMs ($n=12$) had approximately 50 % higher plasma concentrations of morphine and its glucuronides compared with EMs ($n=11$) after administration of a single dose of 30 mg codeine [33]. Only half of the *CYP2D6* EMs felt sedation from the codeine compared to 91 % of the

CYP2D6 UMs. In 2009, Lotsch and colleagues conducted a study in 57 healthy Caucasians to determine if morphine formation from codeine could be predicted prior to codeine administration by using *CYP2D6* genotype- and phenotype-based prediction systems [34]. Most subjects (87.5 %) with low morphine production from codeine were correctly identified. However, satisfactory prediction (87.5 %) of high morphine formation was only achieved when combining genotype with phenotyping. There have been multiple case reports of life-threatening adverse events or fatalities in *CYP2D6* UMs [36–40]. The data from these pharmacogenetic studies suggest that implementation of pharmacogenetic testing for *CYP2D6* prior to codeine therapy could improve efficacy and reduce the incidence of drug toxicity if done in combination with therapeutic drug monitoring of morphine production.

2.3.2 Other Genetic and Nongenetic Factors

CYP2D6 is not the only polymorphic gene involved in the codeine and morphine metabolic pathway. Approximately 70 % of morphine is glucuronidated to morphine-3- and morphine-6-glucuronide, primarily by UGT2B7. The evidence concerning the influence of UGT2B7 polymorphism on codeine and morphine metabolism is conflicting and inconclusive. The most well studied SNP in UGT2B7 (UGT2B7*2) did not affect morphine metabolism in vitro [41, 42]. Other studies have identified additional variants that have an impact on morphine metabolism; however, these studies have not been reproduced in separate cohorts. It is possible that recently identified variants that alter mRNA splicing of UGT2B7 could significantly impact the codeine and morphine pathway [43].

Codeine is metabolized to norcodeine by CYP3A4. CYP3A4 is responsible for the metabolism of approximately 50–60 % of pharmaceuticals used today and is also important for the metabolism of steroid hormones. There are several known genetic variants in CYP3A4, but none of them have been shown to cause a phenotypic change in drug metabolism. However, coadministration of drugs that are CYP3A4 substrates, inducers or inhibitors can affect flux through the codeine pathway. In one case report of a life-threatening opioid intoxication, the authors attributed the observed toxicity to not only CYP2D6 ultrarapid metabolism but also inhibition of CYP3A4 activity by other medications [14]. Further studies in a larger study sample are required to determine the effects of coadministration of drugs that act on CYP3A4 for the metabolism of codeine.

The efflux transporter P-glycoprotein (P-gp), encoded by the ATP-binding cassette BI (ABCB1)/multiple drug resistance 1 (MDR1) gene, is responsible for the transport of many opioids, including morphine-3-glucuronide and morphine-6-glucuronide across the blood–brain barrier. Several studies suggest that the ABCB1:3435C>T variant may influence morphine efflux from the blood–brain barrier and result in variable analgesic response [44]. Also, the same studies have shown that a polymorphism (OPRM1 118A>G) in the μ-opioid receptor, encoded by the opioid receptor μ 1 (OPRM1) gene, is associated with opioid analgesia

[45–47]. In one study, daily opioid doses significantly decreased in a gene dose-dependent manner with the ABCB1 3435C>T variant [45]. The same study found that a tendency toward increased pain and the OPRM1 118A>G variant were associated in a dose dependent manner [45]. Another study found that pain relief variability was significantly associated with both polymorphisms [46].

2.3.3 Analytical Assays and Need for TDM Measurements for Drug Compliance

Serum or plasma assays for prescription opioids are not routinely used for therapeutic drug monitoring in the clinical laboratory. The current testing strategy includes urine screening with immunoassays followed by targeted confirmations with gas chromatography mass spectrometry (GC-MS) or liquid chromatography tandem mass spectrometry (LC-MS/MS). These assays are used to monitor drug use and compliance of prescribed opioids because of the long detection window of the metabolites in urine. However, serum and plasma assays for opioids have many advantages over urine because the opioid level in these matrices is reflective of the patient's clinical state at the time of blood collection. The levels could be used for titrating dose, determining steady-state concentrations or determining the drug and metabolite levels in cases of suspected opioid toxicity. However, the role of TDM in titration and monitoring opioids is not well defined. There are no current practice guidelines that have been established for clinical use. Many opioids meet the general criteria for TDM including a narrow therapeutic index, a poor relationship between drug dose and blood concentration, significant inter-individual variation and a serious consequence for overdosing in some individuals. In a study conducted by Lotsch and colleagues they concluded that in order for codeine therapy to be safe, prediction of morphine formation must be obtained by combining *CYP2D6* genotyping with phenotyping [34]. Phenotyping can account for the other genetic and nongenetic factors listed in Sect. 2.3.2.

Despite the fact that opioid TDM is not routine in the clinical laboratory, there are several published methods for the detection of opioids in serum or plasma using LC-MS/MS [48–52]. LC-MS/MS is capable of detecting polar and thermally labile compounds and thus has advantages over GC-MS for the analysis of opioids. Parent drugs and glucuronide metabolites can be quantitated in the same method. With LC-MS/MS, sample preparation is decreased because there is no need for hydrolysis. These LC-MS/MS methods are commonly used in forensic settings and for research purposes. They include anywhere from 6 to 24 opioids and their metabolites and have lower limits of quantitation down to approximately 0.5 ng/mL for many of the analytes. One method demonstrated an upper limit of quantitation at 2,500 ng/mL [48]. These methods are sufficient for determining opioid and metabolite levels and could be used for TDM in the clinical setting once further studies are conducted to establish appropriate guidelines.

Table 2 Advantages and disadvantages of LC-MS vs. immunoassay (where assays are available) for therapeutic drug monitoring

Attribute	LC-MS	Immunoassay
Analytical sensitivity	Highest	Sufficient for some tests
Analytical specificity	Very high	Questionable, antibody dependent
Operator expertise	High	Low
CLIA test category	Highly complex	Moderately complex
Instrument costs	High	High
Reagent costs	Low	High
Commercial availability	Lab developed test	Unavailable for most drugs of interest
Turnaround time	30–60 min	10–20 min
Random access	Limited	Widely practiced
Multiplex capability	Available	Not possible without multiple assays

3 Analytical Testing Platforms for Therapeutic Drug Monitoring

Pharmacogenomic testing for routine clinical practice is still in its infancy. Relabeling mandates and black box warnings issued by the Food and Drug Administration has accelerated the clinical interest in pharmacogenomic testing for select drugs. Critics to routine implementation have argued that genetic testing only accounts for some of the variability in drug response or prediction of adverse events. Therefore, other predictive phenotypic tools are needed, such as functional testing and therapeutic drug monitoring. The debate regarding the clinical significance of genotyping vs. phenotyping will continue for years to come. Each approach has its advantages and disadvantages. It is likely that optimal patient management will require a combination of both approaches. However, doing both testing may not be a cost-effective strategy unless it can be shown that the improvements in clinical outcomes justify additional costs. If therapeutic monitoring is important for the drugs discussed in this chapter, LC-MS appears to be the analytical method of choice. LC-MS enables detection of parent and metabolite concentration in the same analytical assay. Specific immunoassays for these and other drugs of pharmacogenomics interest are not commercially available. The analytical sensitivity of LC-MS is superior to immunoassays. Table 2 summarizes some of the advantages and disadvantages differences between these two analytical approaches for a variety of attributes.

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