Title

Identification of Cytochrome P450 Polymorphisms in Burn Patients and Impact on Fentanyl Pharmacokinetics: A Pilot Study

Journal

Journal of Burn Care & Research, 40(1)

ISSN

1559-047X

Authors

Grimsrud, Kristin N

Ivanova, Xenia

Sherwin, Catherine M

et al.

Publication Date

2019

DOI

10.1093/jbcr/iry053

Peer reviewed

Identification of Cytochrome P450 Polymorphisms in Burn Patients and Impact on Fentanyl Pharmacokinetics: A Pilot Study

Kristin N. Grimsrud, DVM, PhD,* Xenia Ivanova, BS,* Catherine M. Sherwin, PhD, MSc, FCP,† Tina L. Palmieri, MD, FACS,‡ and Nam K. Tran, PhD, HCLD (ABB), FACB*

Pain management is critical for burn care. Unfortunately, interindividual variation in pharmacokinetics (PK) due to burn hypermetabolism and genetic polymorphisms can lead to treatment failures in this at-risk population. Analgesics may be affected by genetic polymorphisms affecting cytochrome P450 (CYP) drug metabolizing enzymes. Fentanyl is a common opiate primarily metabolized by *CYP3A4* subtypes. Recent studies demonstrate *CYP2D6* variants, affecting fentanyl PK. Functional CYP polymorphisms can significantly alter opiate levels resulting in inadequate analgesia or life-threatening toxicity. The goal of our study was to evaluate fentanyl PK and assess associations with CYP polymorphisms. We obtained samples from the previously banked blood of 13 patients (eight males and five females) with >20% TBSA burns. Mean (*SD*) patient age was 41.7 (14.5) years, and mean burn size was 25.8 (15.3) %TBSA. Plasma fentanyl was quantified, and CYP genotyping was performed. Pharmacokinetic analysis was performed using Monolix software (Lixsoft, France) with a two-compartment population model best-representing fentanyl profiles. Three CYP slow-metabolizing genotypes were identified, which included *CYP2D6*9*, *CYP2D6*29*, and *CYP3A4*1B*. All three patients with variant polymorphisms had increased serum fentanyl concentrations due to impaired clearance. This pilot study supports the need for further research in this topic, and CYP genotyping of individual patients prior to receiving opiate analgesics to inform precision-guided decisions, improve therapeutic efficacy, and, most importantly, increase patient well-being and safety.

Each year more than 486,000 patients are treated for burn injury.[1] Pain is ubiquitous in burns, and pain management in burn patients remains difficult. Burn pain requires a combination of multiple drug classes, with opioids being one of the most efficacious analgesics utilized.[2] Fentanyl, a synthetic opiate, is commonly employed to manage acute pain associated with wound care.[3] However, fentanyl efficacy significantly varies between patients, particularly in burns.[3] Variability in opioid metabolism and elimination may be attributed to multiple physiologic factors including hypermetabolism and genetic factors.[4–7] In recent years, the role of pharmacogenetics (PG) in opioid metabolism has gained renewed interest due to the widespread opioid abuse in the United States.[5] The cytochrome P450 (CYP) family of enzymes are of great PG interest because they are responsible for the majority of drug metabolism and exhibit genetic variance.[7] Usually, these CYP enzymes biotransform endogenous and exogenous molecules to facilitate elimination. However, some compounds are inactivated by CYPs, whereas others such as morphine are bioactivated.[7]

*From the *Department of Pathology and Laboratory Medicine, School of Medicine, University of California, Davis; †Division of Clinical Pharmacology, Department of Pediatrics, School of Medicine, University of Utah, Salt Lake City; and ‡Division of Burn Surgery, Department of Surgery, School of Medicine, University of California, Davis.*

We thank our Clinical Laboratory supporting this study. The project was supported in part by a National Heart Lung and Blood Institute Emergency Medicine K12 (no. 5K12HL108964-05) scholarship.

The authors have no disclosures to report.

Address correspondence to Kristin N. Grimsrud, DVM, PhD, Department of Pathology and Laboratory Medicine, University of California, Davis, School of Medicine, 1 Shields Ave., Davis, CA, 95616 . Email: kngrimsrud@ucdavis.edu.

doi:10.1093/jbcr/iry053

In addition to genetic variations, CYP activity is also influenced by hypermetabolism, and activity may be induced or inhibited by competing medications.[5,8] These factors are all common in severely burned patients where the hypermetabolic state has been suggested to extend for months following injury. Hypermetabolism upregulates CYP pathways, especially in the liver, and could alter the kinetics of the biotransformation of opioids and other medications.[6] Polypharmacy may also play a role in CYP activity and is highly prevalent in critically ill burned patients who by some estimates are prescribed an average of 40.6 medications per day.[9] Furthermore, compounds such as cannabinoids and methamphetamine, common in burn patients, are known inhibitors of the same CYP pathways used by opioids.[7,10]

The genetics of CYP has been shown to play a significant role in drug metabolism. CYP polymorphisms, for example, have been shown to impact drug therapy, especially in cancer patients. Variants have been identified, with *CYP2D6* and *CYP3A4* being the most clinically significant.[11–13] *CYP3A4*, in particular, is responsible for the metabolism of >50% of medications approved by the United States Food and Drug Administration (FDA).[13] Genetic polymorphisms associated with CYPs result in some variants exhibiting slow activity resulting in toxic accumulation of drug, whereas other variants have increased activity resulting in poor efficacy from low concentrations of drug in circulation.[7] *CYP2D6* polymorphisms occur more frequently and are associated with treatment failures and toxicity for several medications (e.g., tamoxifen, propranolol, opioids).[14] The reported allele frequency of *CYP3A4*22* in Caucasians is 5% to 8%, and 4.3% in African Americans and Chinese.[15] Moreover, *CYP2D6* slow-metabolizing variants are reported to be 10% in Caucasians/Hispanics, 2% in African Americans, and 1% in Asians.[15,16] The

*CYP2D6*2*, **3*, **4*, **5*, **6*, **10*, and **41* have been reported to be more common in Caucasians, whereas **2 and *17* were observed in Africans and **10* and **36* more common in Asians.[16] Several other CYPs and genes (e.g., *2B6*, *2C9*, *2C19*, transporters, receptors, and channels) are gaining more importance as knowledge regarding their clinical impact is discovered.[6,17] Therefore, CYP polymorphisms combined with underlying hypermetabolism found in burn patients may place these patients at risk for opioid treatment failures. The goal of our pilot study is to determine the clinical significance of CYP variants associated with altered fentanyl pharmacokinetics (PK) in severely burned patients.

METHODS

We conducted a pilot observational study using banked ethylenediaminetetraacetic acid and sodium-heparinized plasma samples from our College of American Pathologists (CAP)-accredited biorepository. Samples from adult patients (age ≥ 18 years) with ≥20% TBSA burns who were receiving fentanyl therapy were used for the following study. These specimens were previously collected and de-identified as part of a hospital quality improvement project to validate CYP assays for clinical implementation. These samples were selected based on the criteria above with no specification or bias related to the patient's ethnic background, gender, or other specific patient information.

Fentanyl Analysis

Fentanyl testing was performed on the plasma samples as a send-out test to a Clinical Laboratory Improvement Amendment (CLIA) and CAP-accredited referral laboratory using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS). The assay limit of quantitation was 0.1 ng/ml with an analytical precision of 5.3% at this cutoff. Quantitation was based on isotope dilution via deuterated fentanyl (fentanyl-D_5).

Genotype Analysis

CYP genotyping was also performed on ethylenediaminetetraacetic acid whole-blood samples through a CLIA/CAP-accredited referral laboratory. *CYP2D6* and *CYP3A4* genes were targeted. For *CYP2D6*, two stages were used for testing. Stage 1, the laboratory used a polymerase chain reaction (PCR)-based 5′-nuclease assay to determine the presence of any *CYP2D6* variants. All samples also have copy numbers quantified by a PCR-based 5′-nuclease assay. This first stage of testing allows for the detection of all common *CYP2D6* variants (e.g., **2*, **3*, **4*, **5*, **6*, **7*, **8*, **9*, **10*, **17*, **29*, **35*, **41*), as well as rarer alleles such as **11*, **12*, **14A*, **14B*, and **15* along with any allele duplications or multiplications. Unitary and tandem *CYP2D7-2D6 (*13)* alleles and *CYP2D6-2D7* (e.g., **4N*, **36*, and **68*) alleles can also be detected using this method. Second stage of testing involves sequencing using fluorescent dye-terminator chemistry and is only performed if the first stage of results was ambiguous. Similarly, *CYP3A4* genotyping is also performed using a PCR-based 5′-nuclease assay, however, without the two-stage approach. Again, fluorescently labeled detection probes anneal to the target gene. PCR then amplifies the DNA containing the variant. If the PCR detection probe is an exact match to the target DNA, the 5′-nuclease polymerase degrades the probe, and the reporter dye can fluoresce. Genotypes are assigned based on the allele-specific fluorescent signals that are detected.

Pharmacokinetic Modeling

Population analysis using traditional compartmental modeling estimated the PK parameters. Data were assessed with one-, two-, and three-compartment models with zero-order input, to evaluate the most appropriate model for the patients' data set. Monolix software version 2016R1 (Lixoft, Orsay, France) was used to analyze data using a nonlinear mixed effect modeling approach (MONOLIX, 2011). Estimates of the parameters were generated by computing the maximum likelihood of the estimator without approximation of the model using the simulated annealing version of the SAEM algorithm in combination with the Markov chain Monte Carlo procedure.

Using a population analysis approach (equation 1), for the subject I, the individual parameters were defined by:

$$\theta_i = \theta_{\mathrm{POP}} \cdot e^{n_i} \tag{1}$$

where θ_i is the parameter value for the ith subject, θ_{POP} is the parameter value for the population, n_i is the between-subject variability on the parameter, which is normally distributed around a mean of zero and a variance of w_i^2.

Constant (equation 2), proportional (equation 3), and combined (equation 4) error models were evaluated with each of the structural compartment models, where y is the observation, f is the parameter function of the structural model, b is the error term for the proportional model, and e is a sequence of independent random variables normally distributed with a mean of 0 and variance of 1.

$$y = f + a \cdot e \tag{2}$$

$$y = f + b \cdot f \cdot e \tag{3}$$

$$y = f + (a + b \cdot f)e \tag{4}$$

In addition to CYP genotype, covariates considered for the PK model included TBSA burned, age, sex, weight, temperature, systolic blood pressure, burn type, inhalation injury, and substance abuse. Numerical covariates (age, weight, and %TBSA burned) were logarithmically scaled and median centered. Covariates were first considered in the univariate analysis and included for multivariate analysis if they were found to decrease the objective function value significantly. Covariates were incorporated using stepwise forward addition followed by stepwise backward elimination. A decrease of ≥3.84 ($P <$ .05) in the −2*log likelihood was the criterion for retention in the forward addition, and a decrease of ≥6.63 ($P <$ .01) was required in the backward elimination step.

Determination of Goodness-of-Fit

Graphical evaluation for assessing goodness-of-fit and the numerical assessment of the −2*log likelihood, Akaike information criteria, and Bayesian information criterion were the

primary diagnostic parameters considered for final model selection. The graphs generated for model evaluation included the population and individual predictive concentrations over time, observed data vs the population and individual predictions. The model was also evaluated using graphs of population and individual weighted residuals vs time, population and individual weighted residuals vs predictions and a prediction-corrected visual predictive check.

Statistics

The two-sample *t*-test was used to compare independent means. Repeated-measures analysis of variance was used to compare fentanyl concentrations between wild-type and mutant CYP variants. Post hoc analysis using the Bonferroni test was used to determine at which time point fentanyl concentrations became significantly different between wild-type vs mutant variants. A *P* value of <.05 was considered statistically significant. As needed based on Shapiro–Wilk test for normality, nonparametric statistics were employed with median and range reported.

RESULTS

The study was conducted in accordance with Institutional Regulatory Board regulations. Patient demographic data are summarized in Table 1. Respectively, between CYP mutant vs wild-type patients, the mean (*SD*) age (41 [8.2] vs 43 [16.1] years, $P = .315$), percent TBSA burned (24.0 [9.6] vs 27.2 [16.8] %, $P = .562$), and body weight (87.5 [6.8] vs 92.3 [10.1] kg, $P = .066$) were not significantly different between patients with wild-type vs mutant CYP genotypes. Two of the mutations were identified in male patients, and one mutation was identified in a female patient.

Patients in the data set were identified to have fentanyl administered 70 µg/min continuous rate infusion, and whole-blood samples were collected from indwelling venous catheters. Samples were collected following the beginning of fentanyl administration at 0, 15, 30, and 60 minutes and used for determination of plasma fentanyl concentrations for *CYP3A4* and *CYP2D6* genotyping. Repeated-measures analysis identified fentanyl levels 15 min post-administration were significantly ($P < .001$) higher in the mutant CYP vs the wild-type populations. The mean (*SD*) of fentanyl concentrations at 15 minutes for the mutant CYPs vs wild types are 3.2 (0.2) and 1.5 (0.5) ng/ml, respectively.

Genotyping analysis identified three patients with mutant variants *CYP2D6*9*, *CYP2D6*29*, and *CYP3A4*1B* and exhibiting altered fentanyl kinetics. The remaining ten patients were identified to have the wild-type forms of the *CYP2D6* and *CYP3A4* enzymes with normal activity. The *CYP2D6* and *CYP3A4* mutants had significantly higher fentanyl concentrations than the wild-type patients ($P < .01$). The PK parameter estimates for the patients with the mutant CYPs are presented in Table 2.

Table 1. Demographics summary of study subjects

Patient Parameter	Mean	*SD*
Age (yr)	41.7	14.5
Weight (kg)	86.5	20.7
Male:female	8:5	
%TBSA	25.8	15.3

The PK profiles were biexponential in nature. A two-compartmental model with a constant error model was the best operative for describing the time course of the drug profiles for fentanyl in these adult burn patients. The compartmental model was parameterized using clearance (Cl), intercompartmental clearance (*Q*), and volume of distribution for the central (V1) and peripheral compartment (V2). Sample collection was only targeting the first hour following dosing. Thus, the elimination half-life was not calculated from this sparse data set. Weight and CYP genotype were found to be the only covariates affecting fentanyl PK. The equations used to calculate Cl and V_d, with weight as a covariate, are presented in equations (5) and (6), respectively.

$$\begin{aligned}\mathrm{Log(Cl)} = {} & \log(\mathrm{Cl_{pop}}) + \beta_{\mathrm{Cl}} CYP2D6*9 \\ & + \beta_{\mathrm{Cl}} CYP3A4*1B + + \beta_{\mathrm{Cl}} CYP2D6*29 \\ & + \beta_{\mathrm{Cl*tWeight}} \log(\mathrm{Wt}/81) + \eta_{\mathrm{Cl}}\end{aligned} \tag{5}$$

$$\mathrm{Log}\,(V_{\mathrm{d}}) = \log\left(V_{\mathrm{d\,pop}}\right) + \beta_{V\mathrm{d*tWeight}} \log\left(\mathrm{Wt}/81\right) + \eta_{\mathrm{Vd}} \tag{6}$$

The observed and fitted PK profiles for wild-type patients, and mutant variants are presented in Figure 1. Patients with mutant CYP variants demonstrated a prolonged clearance compared with wild-type variants.

DISCUSSION

In this pilot study, we identified three functional CYP polymorphisms that have been documented to have altered functionality and are clinically impactful.[18–20] Polymorphism *CYP2D6*9* is a G_{2613}–A_{2615} deletion with decreased activity and is the most commonly reported mutant of clinical significance, with a prevalence of 2% in Caucasian and African populations and 3% in Asian populations.[20] The *CYP2D6*29* allele is a G_{3183} to A single-nucleotide polymorphism and has been documented to be prevalent in 20% of the African American population and categorized as a poor metabolizer.[18] The *CYP3A4*1B* is an A_{392} to G transition of the proximal promoter region in this gene and is the most common reported single-nucleotide polymorphism reported for the *CYP3A4* family.[14] It has been reported to have higher expression than the wild-type *CYP3A4* and has been published as a poor metabolism of multiple drugs, e.g., quinine,[20] while also being reported as a rapid metabolizer for other drugs, e.g., tacrolimus.[19,21] Fentanyl clearance was decreased in all three patients that were identified to have CYP polymorphisms. To the authors' knowledge, the present study is the first to demonstrate an association between altered fentanyl kinetics with the identification of CYP polymorphisms in burn patients.

We found that patients carrying the wild-type alleles exhibited the expected decline of systemic fentanyl over time, whereas patients with the mutant variants did not eliminate fentanyl efficiently, thus maintained high circulating

Table 2. Clearance and volume of distribution in wild-type and CYP variants

	Wild-type*	*CYP2D6*9*	*CYP2D6*29*	*CYP3A4*1B*
CL (ml/min)	1,115.6 (1,044.4–1,387.2)	9.13	4.67	5.7
V1 (l)	22.3 (19.4–36.0)	16.3	18.1	16.1
V2 (l)	4.25 (2.30–29.7)	1.31	2.11	1.02

*Wild-type data presented as median (range).

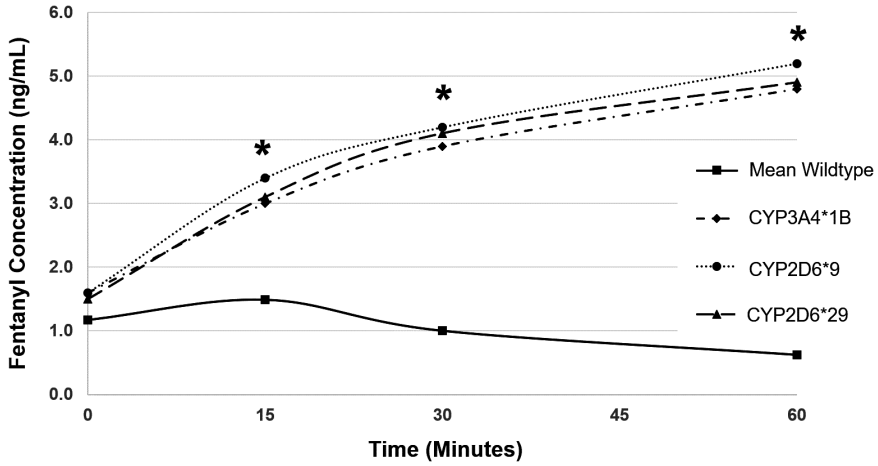

Figure 1. Mean wild-type and individual *CYP3A4*1B*, *CYP2D6*9*, and *CYP2D6*29* fentanyl plasma concentrations plotted on a linear scale at predetermined collection points following the start of a continuous fentanyl constant rate infusion (70 µg/min) with the beginning of the infusion at time 0 minutes. *Timepoint that mutant CYP concentrations were statistically different than wild types ($P < .05$).

concentrations over the monitoring time frame. The present pilot study only analyzed samples over a 60-minute period, thus not fully characterizing in the complete fentanyl profiles, specifically the prolonged elimination, which probably is contributing to possible inaccuracies in the calculated PK parameters (Table 2). Based on the drug profiles presented in Figure 1, it can be predicted that patient fentanyl concentrations continued to increase and had delayed elimination. Comparing the wild-type PK parameters in this study on a per weight basis to a study by Han et al in burn patients, the median CL of 12.46 ml/min/kg and the V1 of 0.258 l/kg are very similar to the published CL (9.0–60.7 ml/min/kg) and V1 (0.27–1.16 l/kg) values.[4] The patients in this study with polymorphisms, as well as other individuals with similar mutations, are at increased risk of fentanyl toxicity and adverse reactions with standard fentanyl dosing regimens. The literature demonstrates the considerable overlap in fentanyl concentrations between therapeutic ranges and toxic ranges, with some individuals experiencing mortality with circulating fentanyl concentrations within the therapeutic range.[22] These observations in overlapping ranges between therapeutic and toxic doses support the need for further studies to be conducted to characterize the covariates affecting efficacy and toxicity of opioid administration, to minimize morbidity and mortality as a result of excessive opioid exposure. To date there have not been any publications fully characterizing the fentanyl toxicity profiles of the polymorphisms identified in this study. However, a study investigating pharmacogenetic implications related to fentanyl deaths did confirm a patient that deceased from fentanyl toxicity was identified to have a *CYP3A4*1B* mutation with concurrent decreased norfentanyl concentrations, providing scientific evidence of the impaired fentanyl metabolism.[23] Other reviews do state that genetic polymorphisms in *CYP3A4* may influence both the efficacy and toxicity of fentanyl.[24] Both *CYP2D6*9* and *CYP2D6*29* are classified as poor metabolizers[16] and have been reported to cause toxic accumulation of circulating drug levels resulting in nausea and vomiting in patients receiving oxycodone, hydrocodone and other opioids.[24,25] Studies correlating the impact of *CYP2D6* poor metabolizing polymorphisms on fentanyl accumulation are lacking and need to be further investigated to identify the potential toxic effects that may result. Increased circulating opioid levels may also raise concerns that a patient may have an increased risk of developing tolerance. Although there has not been literature to date specifically linking these polymorphisms with increased risk for fentanyl tolerance over time, there are reports demonstrating that higher circulating opioid concentrations, thus more drug at the site of action, do result in a cascade of events resulting in opioid receptor internalization and the development of tolerance.[26] As stated by Dumas and Pollack,[26] "Agonist binding, therefore, results in diverse cellular adaptations that mediate antinociception and onset of tolerance", thus one would suspect that a decreased clearance that results in increased circulating levels of agonist would contribute to increasing the susceptibility of the development of tolerance.

Clinical decisions based in part on knowledge of CYP variants are a classic example of precision medicine, but the majority of these clinical applications have involved cancer therapy.[7,27] For example, correlating therapeutic efficacy of tamoxifen in breast cancer patients with *CYP2D6* activity has identified slow-metabolizers with greater risk for tamoxifen-induced toxicity,

while fast-metabolizers exhibit an increased risk for treatment failure and/or tumor recurrence.[27] Outside of cancer therapy, only recently have studies suggested the use of CYP genotyping to improve dosing of common medications, including opioids, for pain management.[7] Although drugs such as fentanyl are heavily metabolized by *CYP3A4*, contrary to historical reports of *CYP2D6* having no role in metabolizing fentanyl, recent studies now suggest otherwise.[12] This phenomenon may be exacerbated during polypharmacy where *CYP3A4* pathways are overwhelmed and forcing fentanyl to be metabolized by *CYP2D6*. The heavy use of opioids in critical care, especially burn patients,[9] highlights the need to evaluate CYP genotypes when prescribing opioids for severe pain management.

As a high-risk population exhibiting significant hypermetabolism following injury and polypharmacy, severe burn patients are particularly susceptible to variable and unpredictable therapeutic responsiveness to opioids. Burn patients have been known to have altered PK profiles due to their hypermetabolic state.[28,29] Kaneda et al demonstrated that burn patients had an expanded volume of distribution of fentanyl compared with nonburned patients, probably attributed to dilutional distribution.[29] Many of the alterations in absorption, distribution, metabolism, and elimination (ADME) are anticipated in burn patients as they often have significant pathophysiological alterations as a result of the burn injury and postburn procedures and treatments.[28] Significant fluid losses and shifts occur, which may be associated with factors such as compartment syndrome, loss of fluids from draining sites and lesions, skin loss contributing to fluid loss from lack of a barrier and wound leakage, blood loss from repeated fasciotomies and other surgeries. Massive fluid resuscitation and frequent blood transfusions greatly alter the expected drug kinetics, often resulting in dilution effects. Moreover, changes in circulating proteins, either from protein loss or dilution from resuscitation may alter the amount of free drug available depending on if the individual compound has high affinity for protein binding. Many burn patients experience significant organ dysfunction (e.g., cardiovascular, renal), as well as hyperimmune and hypermetabolic states, which all can have significant impacts on ADME and overall drug efficacy and toxicity. Literature reports that burn patients are administered an average (*SD*) of 40.6 (20.2) medications prescribed per day in patients with >20% TBSA burns.[9] Common medications include opioids (e.g., fentanyl, hydromorphone) and several compounds that are known to alter CYP activity (e.g., propranolol, fluconazole).[28] Unfortunately, drug dosing in burn patients, including for opioid therapy, is not personalized and does not account for PK, pharmacodynamic (PD), and PG variation, resulting in a high degree of poor efficacy, comorbidities, tolerance, and addiction. Due to the present study being a retrospective analysis with limited patient medical record information, we could not thoroughly evaluate what other confounding variables in our patients, which could also be influencing the fentanyl ADME. However, due to the significant difference in fentanyl concentrations between the three individuals with the polymorphisms and the wild-type patients, the data presented here are highly supportive of these mutations being of clinical significance and prospective study needs to pursue investigating this on a larger scale. Personalized dosing relies on a solid foundation of established PK/PD data. However, a literature review by our institution revealed few, if any, appropriately performed PK/PD studies in burn patients.[28] All of these covariants must be taken into account when considering appropriate dosing regimens in these critically ill burn patients, in addition to potential influences of genetic polymorphisms. The limitations of our study included a small sample size. Although not the goal of our pilot study, PD, and patient outcomes data were not available by nature of our quality database.

CONCLUSIONS

Pharmacogenetic testing may provide a means to improve the precision of opiate dosing in severely burned patients. The PK profiles of fentanyl in patients with CYP polymorphisms are significantly altered and may lead to inappropriate drug levels in vivo. *CYP2D6*9*, *CYP2D6*29*, and *CYP3A4*1B* mutant genotypes presenting as a slow-metabolizer phenotype may be at risk for fentanyl overdose. Further studies are required to determine the clinical impact of CYP polymorphisms in the complex, hypermetabolic, burn patient population.

REFERENCES

1. National Hospital Ambulatory Medical Care Survey. Emergency department summary tables. 2011, accessed 22 Jan. 2015; available from http://www.cdc.gov/nchs/ahcd/web_tables.htm#2011.
2. de Castro RJ, Leal PC, Sakata RK. Pain management in burn patients. Braz J Anesthesiol 2013;63:149–53.
3. Prakash S, Fatima T, Pawar M. Patient-controlled analgesia with fentanyl for burn dressing changes. Anesth Analg 2004;99:552–5, table of contents.
4. Han T, Harmatz JS, Greenblatt DJ, Martyn JA. Fentanyl clearance and volume of distribution are increased in patients with major burns. J Clin Pharmacol 2007;47:674–80.
5. Elmallah RK, Ramkumar PN, Khlopas A, et al. Postoperative pain and analgesia: is there a genetic basis to the opioid crisis? Surg Technol Int 2018;32:306–14.
6. Morgan ET. Impact of infectious and inflammatory disease on cytochrome P450-mediated drug metabolism and pharmacokinetics. Clin Pharmacol Ther 2009;85:434–8.
7. Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 2009;41:89–295.
8. Tannenbaum C, Sheehan NL. Understanding and preventing drug-drug and drug-gene interactions. Expert Rev Clin Pharmacol 2014;7:533–44.
9. Godwin Z, Lima K, Greenhalgh D, Palmieri T, Sen S, Tran NK. A retrospective analysis of clinical laboratory interferences caused by frequently administered medications in burn patients. J Burn Care Res 2016;37:e10–7.
10. Kapur BM, Lala PK, Shaw JL. Pharmacogenetics of chronic pain management. Clin Biochem 2014;47:1169–87.
11. Feierman DE, Lasker JM. Metabolism of fentanyl, a synthetic opioid analgesic, by human liver microsomes. Role of CYP3A4. Drug Metab Dispos 1996;24:932–9.
12. Shu-Biao W, Xian-Hui Y, Hong-Guang F, et al. Impact of CYP2D6 polymorphisms on postoperative fentanyl analgesia in gastric cancer patients. Genet Test Mol Biomarkers 2015;19:248–52.
13. Topletz AR, Dennison JB, Barbuch RJ, Hadden CE, Hall SD, Renbarger JL. The relative contributions of CYP3A4 and CYP3A5 to the metabolism of vinorelbine. Drug Metab Dispos 2013;41:1651–61.
14. Dahabreh I, Terasawa T, Castaldi P, Trikalinos TA. CYP2D6 testing to predict response to tamoxifen in women with breast cancer: pharmacogenomic. PLoS Curr 2010;2:RRN1176.
15. Bernard S, Neville KA, Nguyen AT, Flockhart DA. Interethnic differences in genetic polymorphisms of CYP2D6 in the U.S. population: clinical implications. Oncologist 2006;11:126–35.

16. Teh LK, Bertilsson L. Pharmacogenomics of CYP2D6: molecular genetics, interethnic differences and clinical importance. Drug Metab Pharmacokinet 2012;27:55–67.
17. Preissner SC, Hoffmann MF, Preissner R, Dunkel M, Gewiess A, Preissner S. Polymorphic cytochrome P450 enzymes (CYPs) and their role in personalized therapy. PLoS One 2013;8:e82562.
18. Wennerholm A, Johansson I, Hidestrand M, Bertilsson L, Gustafsson LL, Ingelman-Sundberg M. Characterization of the CYP2D6*29 allele commonly present in a black Tanzanian population causing reduced catalytic activity. Pharmacogenetics 2001;11:417–27.
19. van der Weide J, Hinrichs JW. The influence of cytochrome P450 pharmacogenetics on disposition of common antidepressant and antipsychotic medications. Clin Biochem Rev 2006;27:17–25.
20. Marwa KJ, Schmidt T, Sjögren M, Minzi OM, Kamugisha E, Swedberg G. Cytochrome P450 single nucleotide polymorphisms in an indigenous Tanzanian population: a concern about the metabolism of artemisinin-based combinations. Malar J 2014;13:420.
21. Shi WL, Tang HL, Zhai SD. Effects of the CYP3A4*1B genetic polymorphism on the pharmacokinetics of tacrolimus in adult renal transplant recipients: a meta-analysis. PLoS One 2015;10:e0127995.
22. Martin TL, Woodall KL, McLellan BA. Fentanyl-related deaths in Ontario, Canada: toxicological findings and circumstances of death in 112 cases (2002–2004). J Anal Toxicol 2006;30:603–10.
23. Jin M, Gock SB, Jannetto PJ, Jentzen JM, Wong SH. Pharmacogenomics as molecular autopsy for forensic toxicology: genotyping cytochrome P450 3A4*1B and 3A5*3 for 25 fentanyl cases. J Anal Toxicol 2005;29:590–8.
24. Holmquist GL. Opioid metabolism and effects of cytochrome P450. Pain Med 2009;10 Suppl 1:S20–9.
25. Foster A, Mobley E, Wang Z. Complicated pain management in a CYP450 2D6 poor metabolizer. Pain Pract 2007;7:352–6.
26. Dumas EO, Pollack GM. Opioid tolerance development: a pharmacokinetic/pharmacodynamic perspective. AAPS J 2008;10:537–51.
27. Singh MS, Francis PA, Michael M. Tamoxifen, cytochrome P450 genes and breast cancer clinical outcomes. Breast 2011;20:111–8.
28. Steele AN, Grimsrud KN, Sen S, Palmieri TL, Greenhalgh DG, Tran NK. Gap analysis of pharmacokinetics and pharmacodynamics in burn patients: a review. J Burn Care Res 2015;36:e194–211.
29. Kaneda K, Han TH. Comparative population pharmacokinetics of fentanyl using non-linear mixed effect modeling: burns vs. non-burns. Burns 2009;35:790–7.