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Ethnic and genetic factors in methadone pharmacokinetics: A population pharmacokinetic study[☆]

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Abstract

Background—Treatment of opiate use disorders with methadone is complicated by wide interindividual variability in pharmacokinetics. To identify potentially contributing covariates in methadone pharmacokinetics, we used population pharmacokinetic modeling to estimate clearance (CL/F) and volume of distribution (V/F) for each methadone enantiomer in an ethnically diverse methadone maintained population.

Methods—Plasma levels of the opiate-active *R*-methadone and opiate-inactive *S*-methadone were measured in 206 methadone maintained subjects approximately two and twenty-three hours after a daily oral dose of racmethadone. A linear one-compartment population pharmacokinetic model with first-order conditional estimation with interaction (FOCE-I) was used to evaluate methadone CL/F and V/F . The influence of covariates on parameter estimates was evaluated using stepwise covariate modeling. Covariates included ethnicity, gender, weight, BMI, age, methadone dose, and 21 single nucleotide polymorphisms in genes implicated in methadone pharmacokinetics.

Results—In the final model, for each enantiomer, Hmong ethnicity reduced CL/F by approximately 30% and the rs2032582 (*ABCB1* 2677G > T/A) GG genotype was associated with a 20% reduction in CL/F . The presence of the rs3745274 minor allele (*CYP2B6* 515G > T)

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Contributors

All authors had access to the data utilized in production of this manuscript. All authors have read and approve the final version of the manuscript. GB wrote the manuscript. GB, RJS, and RCB designed the research. GB, SL, and RCB performed the research. GB and RCB analyzed the data.

Conflict of interest

No conflict declared.

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reduced CL/*F* by up to 20% for *S*-methadone only. A smaller effect of age was noted on CL/*F* for *R*-methadone.

Conclusion—This is the first report showing the influence of the rs2032582 and rs3745274 variants on methadone pharmacokinetics rather than simply dose requirements or plasma levels. Population pharmacokinetics is a valuable method for identifying the influences on methadone pharmacokinetic variability.

Keywords

Ethnicity; Genetics; Hmong; Methadone; Pharmacogenetics; Population pharmacokinetics

1. Introduction

The misuse of and dependence on opiates is associated with significant morbidity and mortality through overdose and infectious diseases transmitted by injection drug use (Degenhardt et al., 2009). For more than 40 years, the long-acting synthetic opioid methadone has played a central role in the treatment of opiate dependence (Kleber, 2008).

Despite its effectiveness in the treatment of opiate use disorders, methadone is often difficult to use due to its highly variable pharmacokinetics. Estimates of methadone's clearance, volume of distribution, and half-life range from 5.9–13 l/h, 189–470 l, and 15–207 h, respectively (Eap et al., 2002). This difficulty is apparent in the significant rise in methadone associated mortality primarily seen when prescribed for pain by physicians who likely are less familiar with this variability than physicians within highly regulated methadone maintenance settings (Center for Substance Abuse Treatment, 2007). While training in safe prescribing strategies for methadone has resulted in reduced mortality, methadone remains a medication which has highly variable pharmacokinetics making it difficult to devise standard dosing regimens informed by therapeutic drug monitoring (Strang et al., 2010).

Methadone is a racemic mixture whose *R*-enantiomer provides the therapeutic effect at muopioid receptors, while both the *R* and *S*-enantiomers are weak *N*-methyl-D-aspartate (NMDA) receptor antagonists (Eap et al., 2002). Most studies of methadone pharmacokinetics have evaluated only total methadone levels; however, it appears that there is also variability in pharmacokinetics within and between enantiomers (*R*-methadone: clearance 4–9.6 l/h, volume of distribution 96–469 l, half-life 24–48 h; *S*-methadone: clearance 7.7–20 l/h, volume of distribution 259–273 l, half-life 20–40 h), which may complicate interpretation of studies assessing the pharmacokinetics of total methadone only.

Population pharmacokinetics (POPPK) is a useful and valid approach toward quantifying drug exposure–clinical response relationships (Food and Drug Administration, 1999; Sheiner and Ludden, 1992). Unlike traditional pharmacokinetic studies, which gather dense data that assess individual variability in drug kinetics, POPPK can use sparse data to model measures of drug exposure and identify factors (e.g., ethnicity, gender, age, weight) that influence variability in drug concentrations across populations (Sheiner et al., 1977).

While the POPPK approach has been validated for methadone maintained individuals there are no studies from large or diverse populations (Foster et al., 2004; Rostami-Hodjegan et

al., 1999; Wolff et al., 1997). In fact, over 90% of the subjects in previous POPPK studies of methadone were Caucasian and none of the studies were conducted within a United States population. With larger more diverse sample sizes, variables that contribute to methadone pharmacokinetics (e.g., ethnicity) and treatment outcome may be identified. Based on our previous observations that methadone maintained ethnic Hmong from Laos are on a lower mean dose of methadone yet achieve greater treatment response than do non-Hmong attending the same clinic (Bart et al., 2012), we hypothesized that POPPK could detect decreased methadone clearance in Hmong compared to non-Hmong.

2. Methods

2.1. Subjects

Methadone maintained patients enrolled in a single urban outpatient addiction medicine clinic were recruited into two separate POPPK studies: a cross-sectional study requiring patients to have been on methadone for at least two months without dose change during the previous five days and a prospective study that recruited patients during their first week on methadone and followed them at 1, 3, 6, and 12 months. The prospective study was closed to enrollment after the first 14 subjects due to slow accrual and these subjects were followed as per protocol but once they met inclusion criteria for the cross-sectional study, their prospective data were combined with the cross-sectional subjects in creating the population pharmacokinetic model.

As per Federal criteria, all subjects on methadone maintenance were at least 18 years of age and had met Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for opiate dependence of at least one year duration prior to initiating methadone. Subjects were excluded if they were unable or unwilling to provide informed consent, had decompensated liver disease, were in the second or third trimester of pregnancy, or were taking medications known to alter methadone pharmacokinetics (specifically, phenytoin, rifampin, or highly active antiretroviral therapy for HIV). The study was approved by the Human Subjects Research Committee of the Hennepin County Medical Center and conducted in accordance with the Helsinki Declaration of 1975 (as revised in 1983). Because data included sensitive psychiatric and drug related information a Certificate of Confidentiality was obtained from the National Institutes of Health National Institute on Drug Abuse. The Hmong speaking population was not literate in English or written Hmong, however, consent forms were translated into Hmong and then read to patients by native Hmong speakers in the presence of a study coordinator who could answer questions about the protocol.

Two hundred twenty-four subjects consented to participate in this study. Six subjects did not show up for their study date, two withdrew consent, seven had poor venous access, and three were excluded because their methadone dose had changed within five days of study. Thus, 206 subjects and 441 plasma samples (including prospectively collected specimens from 13 subjects) are included in the analyses.

2.2. Procedures

After signing informed consent, 10 ml of venous blood was drawn into lithium heparin blood collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) approximately 22–24 h after the previous day's dose of methadone (i.e., roughly trough) and then again 2–4 h after taking their daily methadone dose (i.e., roughly peak). With separate consent, two 10 ml venous blood samples (15% EDTA tubes, Tyco Healthcare Group LP, Mansfield, MA) were collected for DNA isolation and analysis. In a few instances when venous access for one of these time points could not be obtained, a peak or trough sample was taken on a later date. Date, time, and amount of each methadone dose were obtained from a real-time medication dispensing software system (Methasoft, Netalytics, Greer, SC) and dates and times of all blood samples were maintained in a Microsoft Access database. All doses of methadone were consumed under direct supervision on the day of study and more than 95% of the previous day doses were also directly observed (exceptions were for Sunday doses when patients were studied on a Monday).

Subject weight and height were collected on a standard clinical scale (Seca Model 700, Seca Corporation, Hanover, MD) for body mass index (BMI) calculation as weight in kilograms/height in meters². Subjects self-identified ethnicity and medications they were taking. Subjects also provided a urine specimen for drug testing and completed the Structured Clinical Interview for DSM-IV (First et al., 1998) to confirm opiate dependence diagnosis and the Symptom Checklist-90 to evaluate for ongoing psychopathology (Derogatis et al., 1973). All interviews were conducted by a single trained master-level research coordinator. For Hmong subjects, interviews were conducted with the assistance of an interpreter knowledgeable in medical and drug use terminology.

2.3. Assays

Blood samples were placed on ice and, within 45 min of being drawn, were centrifuged at 2000 ×g for 15 min at 4° centigrade for plasma separation. Plasma was immediately stored in 2.0 ml Nunc Cryotubes (Thermo Fisher Scientific, Rochester, NY) at –80° centigrade until analyzed. Plasma levels of each methadone enantiomer were determined using an LC–MS/MS protocol adapted from Liang et al. (Liang et al., 2004).

LC–MS/MS was performed using a TSQ Quantum Classic LC–MS/MS (Thermo Scientific, Waltham, MA) with Agilent 1200 HPLC (Agilent, Santa Clara, CA) and a Chiral-AGP column (5 cm × 2.0 mm, 5 µm particle size, Regis Technologies, Morton Grove, IL). Calibration and quality control using *R*- and *S*- methadone and their D₃-isotope counterparts (Cerilliant, Round Rock, TX) revealed an assay lower level of quantitation of 2.75 ng/ml for *R*-methadone and 2.25 ng/ml for *S*-methadone and a linear range of detection measured between 2.75–687 ng/ml and 2.25–565 ng/ml, respectively. Although not tested beyond these ranges, Liang et al. (2004) found this methodology to be linear up to 1000 ng/ml for each enantiomer. Between and within assay variability percent coefficient of variation were below 6% for both enantiomers.

Urine specimens collected on day of study were analyzed for amphetamine, benzodiazepine, barbiturates, cocaine, and opiates using a commercial immunoassay (EMIT, Beckman, Brea,

CA). The presence of methadone metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in urine was also determined (CEIDA, Microgenics, Fremont, CA). All urine drug screening was performed on site in a Clinical Laboratory Improvement Amendments (CLIA) and College of American Pathologists certified laboratory at the Hennepin Faculty Associates (Minneapolis, MN).

2.4. Genetic analysis

Whole blood drawn for genetic analysis was immediately shipped to the Rutgers Cell and DNA Repository for DNA extraction. Purified DNA (mean concentration 116 ng/μl) was shipped to the University of Minnesota for genotyping. Based on published literature, twenty-one single nucleotide variants across a number of genes with known functional effect or that have been explored in previous methadone or opiate use disorder studies were selected for analysis using Sequenom's (San Diego, CA) iPLEX Gold reaction and MassARRAY System for MALDI-TOF (matrix-assisted laser desorption ionization—time of flight) mass spectrometry based genotyping¹.

2.5. Population pharmacokinetic analysis

For determination of population pharmacokinetics, a nonlinear mixed-effects modeling (NONMEM) approach was used with a sparse sampling design including two time points, roughly 2–4 h and 22–24 h after once daily methadone dosing. *R*- and *S*-methadone were analyzed separately. A one-compartment model with first order absorption and elimination (ADVAN2 TRANS2) was used with the NON-MEM 7.2 and PDx-Pop 5.0 (both Icon Development Solutions, Ellicott City, MD) software packages installed on a Gateway computer running the Intel 8 fortran compiler, Xpose 4.3.2 (<http://www.xpose.sourceforge.net>), and R 2.13.0 (The R Foundation for Statistical Computing, Vienna, Austria). While methadone displays a two-compartment pharmacokinetic profile, modeling this is not feasible with only two time points. However, model mis-specification in this instance would affect only estimates of *K_a*, which we fixed, and would not bias estimates of *CL/F* or *V/F* (Kowalski and Hutmacher, 2001). A first-order conditional estimation with interaction (FOCE-I) was used for all analyses. Based on existing literature, the absorption constant *K_a* was fixed at 1.5 (Wolff et al., 1993), thus the model estimated *CL/F* and *V/F*. The interindividual variability (IIV) was modeled using exponential models for *CL/F* and *V/F*, and residual unexplained variability (RUV) was modeled using a proportional error model. The sparse sampling design inherent with these data was able to support estimations of IIV for *V/F* for both enantiomers, but due to the relative imprecision of the estimates and large shrinkages covariate testing was not conducted on *V/F*.

A base pharmacokinetic model was developed without covariate effects for each enantiomer. An automated stepwise covariate model (SCM) approach was implemented using Pearl-speaks-NONMEM (PsN) to identify candidate covariate effects on *CL/F*. Covariates considered in the SCM analysis were chosen based on biological plausibility and population frequency and included gender, ethnicity (categorical as Caucasian, African

¹See the Supplementary materials for variant identification, further methods, and primer design by accessing the online version of this paper at <http://dx.doi.org> and by entering doi:.

American, American Indian, Hmong, Hispanic), age (scaled to 48 years), weight (scaled to 70 kg), body mass index (scaled to 25 kg/m²), prospective study participation with repeat measures, Global Severity Index score of the SCL-90, urine toxicology result, and genotype of twenty-one different single nucleotide polymorphisms across the *ABCB1*, *CYP3A4*, *CYP2B6*, *CYP2D6*, *CYP2C19*, and *CYP1A2* genes². Since the rs2032582 (*ABCB1* 2677) variant is triallelic, analyses were based on the following three groups: GG, AG or GT, and TT, AT, or AA. Since the A allele is rare and its population estimates are not present in HapMap, it is interesting to note that the A allele was identified in 12 Hmong, 4 Caucasian, 2 American Indian, and 1 Hispanic subjects³.

Because the use of medications known to interact with methadone was an exclusion criterion and inspection of enrolled subject medication lists did not include any specific classes of medication that would plausibly alter methadone pharmacokinetics, co-medication was not included as a covariate. Initial analysis found no difference in pharmacokinetic parameter estimates between Caucasian, African American, and American Indian subjects so data are presented as a combined group throughout the manuscript⁴.

The SCM regressed each covariate on *CL/F* using a power model in a univariate manner. The covariate providing the most significant decrease in objective function value (OFV; a goodness of fit measure similar to a sum of squares) was added into the model and all remaining covariates were tested again. In this forward selection stage, a drop in the OFV of 3.8 was considered significant ($p < 0.05$, chi-square with 1 degree of freedom). This process continued until no covariates were significant. Covariates in this full model were next deleted in a stepwise manner. Any covariate whose deletion from the model resulted in a change in OFV greater than 6.6 ($p < 0.01$) was retained in the model. The reduced final model was defined when no additional covariates could be deleted from the model.

Model building was performed separately for each enantiomer.

2.6. Model qualification

Final model parameter estimates and their 95% confidence intervals were qualified by reestimation using a nonparametric bootstrap approach. NONMEM was used to generate 1000 bootstrap datasets with replacement and fit the final model to each of these data sets. Parameter estimates were rank ordered and the values at the 2.5-and 97.5-percentiles of the rank order were used as the lower and upper bounds of the bootstrap 95% confidence interval. In addition, a visual predictive check (VPC) was performed to assess if data simulated using the final parameter estimates were in agreement with the observed concentrations. Because the study design included sparse sampling that didn't require strict times for blood draws, a standard binning approach was used. Peak concentrations within the 0–5 h time frame were binned, as were trough concentrations in the 21–28 h period.

²See the Supplementary materials for specific variants by accessing the online version of this paper at <http://dx.doi.org> and by entering doi:.

³See the Supplementary materials for genotype by ethnicity information by accessing the online version of this paper at <http://dx.doi.org> and by entering doi:.

⁴See the Supplementary materials for *CL/F* by ethnicity data by accessing the online version of this paper at <http://dx.doi.org> and by entering doi:.

Concentrations between these two bins were too sparse to allow any qualification with a VPC.

3. Results

Data from 206 methadone maintained subjects and 441 methadone plasma concentrations were included in this study. Baseline characteristics between Hmong and non-Hmong subjects are presented in Table 1A (Table 1B provides a breakdown of the non-Hmong group). Plasma methadone concentrations were generally measured 2–4 h and 24–25 h after methadone dosing.

3.1. R-Methadone

Base model parameter estimates for CL/F and V/F were 7.6 l/h and 310 l, respectively. R -methadone half-life, therefore, was 28.3 h. Following the structured covariate modeling approach, covariates on CL/F included Hmong ethnicity, age, and the rs2032582 GG genotype. Diagnostic plots for the final model are presented in Fig. 1. Due to concern that Hmong race may be collinear with weight and BMI and thus bias CL/F , visual inspection of diagnostic plots showed that there was no correlation between weight and clearance in the study population (data not shown). There was also no difference in the milligram per kilogram dose requirement between Hmong and non-Hmong, 0.89 mg/kg (SD 0.32) and 0.98 mg/kg (SD 0.45), $p = 0.1$, respectively.

3.2. Model qualification

From 1000 bootstrap simulations, median parameter estimates from the bootstrap were comparable to estimates from NONMEM and varied by less than 10% from those in the final model (see Table 2 for results).

The final model for R -methadone is represented in the following equations:

$$CL/F = 7.6 \text{ l/h} \times (\text{age}/48)^{-0.359} \times 0.820 \text{ (if rs2032582 GG)} \times 0.713 \text{ (if Hmong)}$$

$$V/F = 310 \text{ l}$$

$$K_a \text{ (h}^{-1}\text{)} = 1.5 \text{ (fixed)}$$

We halved and doubled our estimate of K_a and did not obtain significantly different parameter estimates (data not shown).

3.3. Visual predictive check

Information from the VPC is presented in Fig. 2a as 5th, 50th (median), and 95th percentile of R -methadone concentrations. The results indicate that the simulated concentrations from the VPC are in agreement with the observed data. The 5th, 50th (median), and 95th

percentiles of the observed data (binned) fall within the 95% prediction intervals of the corresponding simulated data (Fig. 3).

3.4. S-Methadone

Base model parameter estimates for CL/F and V/F were 10.2 l/h and 229 l, respectively. *S*-methadone half-life, therefore, was 15.6 h. Following the SCM method, informative covariates on CL/F included Hmong ethnicity, the rs2032582 GG genotype, and rs3745274 minor allele (*CYP2B6* 516G > T).

3.5. Model qualification

From 1000 bootstrap simulations, median parameter estimates from the bootstrap were comparable to estimates from NONMEM and varied by less than 10% from those in the final model with the exception that the estimate of interindividual variability on V/F was 20% greater in the bootstrap estimate (see Table 3 for results).

The final model for *S*-methadone is represented in the following equations:

$$CL/F = 10.21/h \times 0.787 \text{ (if rs2032582 GG)} \times 0.810 \text{ (if rs3745274 min or allele)} \times 0.676 \text{ (if Hmong)}$$

$$V/F = 229 \text{ l}$$

$$K_a \text{ (h}^{-1}\text{)} = 1.5 \text{ (fixed)}$$

We halved and doubled our estimate of K_a and did not obtain significantly different parameter estimates (data not shown).

3.6. Visual predictive check

The 5th, 50th (median), and 95th percentile *S*-methadone concentrations obtained from VPC simulations are shown in Fig. 2b. As with *R*-methadone, the results indicate the simulated data are consistent with the observed data.

4. Discussion

This study used population pharmacokinetics to show that, compared to non-Hmong, Hmong have a lower relative apparent oral clearance of methadone. This result provides pharmacokinetic support to our current and previous observation that Hmong patients require lower methadone doses than non-Hmong patients (Bart et al., 2012). We also found an influence of increasing age, and the major rs2032582 GG genotype on reducing *R*-methadone clearance. The rs2032582 GG genotype and the rs3745274 minor allele were associated with reduced *S*-methadone clearance. While an allele-dose effect for rs3745274 and increasing age both demonstrated some signal for reducing *S*-methadone CL/F , they did not maintain statistical significance in the backward elimination stage (data not shown).

To our knowledge, this is the first report of the influence of ethnicity and genetic variants on methadone pharmacokinetic parameters. While prior studies have found associations between genetic variants and methadone dose or plasma levels, the interpretation of dose–response relationships is complicated because a combination of both pharmacokinetic and pharmacodynamic factors intervene between dose and response, and interpretation of a plasma level at any given time is dependent on dose. Since a pharmacokinetic parameter such as clearance is usually independent of dose, our findings can be used to predict concentration at any time-point for any patient regardless of dose and are not confounded by pharmacodynamic factors as can occur in studies using dose or plasma levels alone.

Our overall parameter estimates of CL/F are similar to previous population pharmacokinetic studies of methadone (Foster et al., 2004; Rostami-Hodjegan et al., 1999). Importantly, one of these studies performed parallel analysis of selected sparse samples with a full set of dense sampling data to validate population pharmacokinetic parameter estimates against traditional pharmacokinetic measures within a single population (Foster et al., 2004). Parameter estimates for *R*-methadone in Foster et al. (2004), were CL/F of 8.7 l/h and V/F of 597 l whereas those for *S*-methadone were CL/F of 8.3 l/h and V/F of 345 l. The sparseness of time points obtained in this study limited our ability to estimate V/F and to test a biexponential model; however, this form of model mis-specification should not bias our estimates of CL/F (Kowalski and Hutmacher, 2001).

Our finding that pharmacokinetic parameters were influenced by genetic variants is of particular interest. For example, the *ABCB1* gene encodes the *P*-glycoprotein efflux transporter located in the intestinal lumen, blood brain barrier, and kidney. Methadone is a substrate for this transporter and, therefore, functional variants of this gene could influence methadone pharmacokinetics. In fact, knockout studies in mice lacking *ABCB1* show higher brain levels of methadone and heightened methadone-induced analgesia compared to wild-type mice (Thompson et al., 2000; Wang et al., 2004). In humans, *P*-glycoprotein inhibition with quinidine increased plasma methadone concentrations and effect on pupil size following oral administration but had no effect on methadone levels or pupil size following intravenous methadone, indicating that in humans *P*-glycoprotein does not significantly influence methadone access to the brain (Kharasch et al., 2004).

Genetic variants of *ABCB1* have been associated with methadone dose requirements, although results are mixed (Coller et al., 2006; Hung et al., 2011; Levran et al., 2008). Levran et al. (2008) did not find a significant effect of rs2032582 variants on methadone dose in stabilized methadone patients but when this variant was included with the rs1045642 (*ABCB1* 3435C > T) and rs1128503 (1236 C > T) variants in a haplotype analysis, patients with a TT or GT genotype required higher methadone doses than those without these genotypes. Another study found that a five variant haplotype that included rs2032582 was significantly associated with methadone dose, with patients having one or two haplotypes containing the rs2032582 major allele requiring higher methadone doses (Coller et al., 2006). While these studies looked only at methadone dose, Crettol et al. (2006) also evaluated methadone plasma levels in relation to the rs2032582 variant. Carriers of the G allele had significantly higher trough levels of *R*-methadone with no effect on *S*-methadone or peak levels of either enantiomer. In a three SNP haplotype analysis, the rs2032582 major

allele was associated with a 1.2 fold increase in plasma levels of both *R*- and *S*-methadone. Our findings are limited, in that we did not perform haplotype analyses to determine whether they are due to the rs2032582 variant or other linked variants, however, the direction of our observation is consistent with that of Crettol et al. (2006) in that reduced clearance would predict higher plasma levels. Interestingly, while in our study and in HapMap African-Americans are more likely to have the rs2032582 GG genotype, we did not find that African-American ethnicity was a significant predictor of methadone pharmacokinetics indicating that, in this instance, African-American ethnicity is likely a surrogate marker for or collinear with the GG genotype.

There are several reports of differential pharmacokinetic parameters for *R*- and *S*-methadone. Kharasch et al. (2009a, 2009b, 2012) have noted reduced *CL/F* and *V/F* for *S*-methadone compared to *R*-methadone. Foster et al. (2004) did not identify a difference in *CL/F* between the enantiomers but did find that *V/F* was significantly lower for *S*-methadone. Boulton et al. (2001) found opposite results with *CL/F* and *V/F* of *S*-methadone significantly greater than *R*-methadone. Since *S*-methadone is stereoselectively metabolized by CYP2B6, it is of particular interest that we found the rs3745274 variant had a large effect on *S*-methadone clearance (Chang et al., 2011; Gerber et al., 2004; Totah et al., 2007, 2008).

The rs3745274 variant encodes the CYP2B6*6 variant and imparts a poor metabolizer phenotype. In vivo studies show that the variant results in aberrant splicing and reduced protein expression (Hofmann et al., 2008). Although this variant is in strong linkage disequilibrium with at least one other variant (rs2279343, or *CYP2B6* 792A > G), we did not do haplotype analyses. In this instance this may not be a limitation in that Hofmann et al. determined that despite linkage disequilibrium the decrease in protein expression is specifically attributable to the rs3745274 variant (Hofmann et al., 2008; Lang et al., 2001).

A number of studies have linked the rs3745274 variant to alterations in pharmacokinetic parameters. For example, both efavirenz and nevirapine, non-nucleoside reverse transcriptase inhibitors used in the treatment of HIV, exposure are increased in carriers of the rs3745274 minor allele, a result consistent with our observation of decreased *S*-methadone clearance (Gounden et al., 2010; Haas et al., 2004; Lehr et al., 2011). In addition to greater exposure, those with an rs3745274 minor allele were more likely to experience efavirenz induced neurotoxicity (Gounden et al., 2010; Haas et al., 2004). In methadone maintained populations this variant has been associated with lower dose requirements, with higher trough levels of *S*-methadone, and a greater peak trough ratio for *S*-methadone compared to *R*-methadone (Crettol et al., 2006, 2005; Hung et al., 2011).

Since *S*-methadone is the inactive enantiomer, we do not expect a direct clinical effect of the 20% decrease in *S*-methadone clearance for the rs3745274 minor allele carriers. In patients whose methadone levels are being measured for clinical correlates, however, an assay that is not stereoselective would be anticipated to give a misleadingly high level for carriers of the rs3745274 minor allele due to over representation of *S*-methadone. This may explain, in part, the wide interindividual variability seen in several studies of methadone levels. Given that as many as 30–40% of Caucasian and African populations carry at least one rs3745274 minor allele and the confounding nature of this genotype on “total” methadone levels, this

calls to question whether therapeutic drug monitoring using a non-stereoselective assay could be meaningfully interpreted.

While we found Hmong ethnicity to be associated with reduced CL/F , the power to detect the influence of other ethnicities on CL/F may be limited by sample size. We repeated the analyses by combining non-Hmong ethnic groups and did not obtain significantly different results (data not shown). Another limitation was our small set of hypothesis driven genetic data which prevented us from doing larger haplotype analyses or evaluating the role of other genes that could explain the difference in CL/F between Hmong and non-Hmong (e.g., genes encoding methadone binding proteins like alpha 1-acid glycoprotein or CYP 3A5). While we tested for interaction between rs2032582 and each ethnicity and found no significant interaction, we cannot rule out the occurrence of type 2 error.

The Hmong remain a non-admixed ethnic minority and their ethnicity may serve as a surrogate marker for genetic influences on methadone pharmacokinetics. The unique genetic background of the Hmong is supported by microsatellite marker studies performed in other minorities from the same geographic region (Listman et al., 2011, 2007). For example, the prevalence of the rs2032582 minor allele in our Hmong population was lower than that reported for Han Chinese in the HapMap database, whereas our non-Hmong populations had a minor allele prevalence similar to those in HapMap⁵. While there is generally good concordance between self-reported ethnicity and ancestry informative markers used in genotyping (Yang et al., 2005), our study did not use these markers to explore for possible admixture or ethnicity misclassification. Since ethnicity and genetics may be collinear, we cannot rule out the presence of gene $\times$ ethnicity interactions.

Further limitations were that we did not have a large prospectively assessed population that would allow us to detect possible methadone autoinduction. While the Hmong were on methadone on average longer than the non-Hmong subjects, we do not anticipate that this difference in length of time on methadone confounds our results as the mean length of time on methadone in both groups was greater than 1.5 years, well beyond any expected time for autoinduction. Finally, we used limited pharmacodynamic data such as ongoing drug use or SCL-90 scores but did not perform pharmacokinetic–pharmacodynamic (pk–pd) studies using opiate related measures such as pupillary response or symptoms of clinical withdrawal or intoxication.

Reduced methadone CL/F in Hmong is consistent with our previous findings that Hmong reach stabilization on lower methadone doses than non-Hmong patients (Bart et al., 2012). This may indicate unique pharmacogenetic or plasma protein binding influences on methadone in this population. There were also influences on CL/F for age and the rs2032582 GG genotype for *R*-methadone. For *S*-methadone, CL/F was noted to have a comparable decrease for the rs2032582 GG genotype but an age effect was determined to be not significant. In addition, the rs3745274 minor allele was found to decrease *S*-methadone CL/F .

⁵Supplementary materials can be found by accessing the online version of this paper at <http://dx.doi.org> and by entering doi

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.drugalcdep.2014.10.014>.

Fig 1.

Goodness of fit plots for **R**-methadone. Upper panels are the observed individual (left) and population (right) concentrations (ng/ml) versus predicted concentrations (ng/ml). Lower panels are the conditional weighted residuals (CWRES) versus the predicted concentration (ng/ml) (left) and conditional weighted residuals versus time after dose (h) (right).

Fig 2.

Visual predictive check. Information from the VPC is presented as 5th, 50th (median), and 95th percentile of methadone concentrations (ng/ml) ((a) *R*-methadone; (b) *S*-methadone). The raw observed data are presented as open circle symbols. The median of the observations within each of the three bins is presented as the heavy solid line. The dashed lines represent the 5th and 95th percentiles of the observed data in that bin. The gray bars represent the 95% prediction intervals around the 50th (darker) and 5th and 95th (lighter) percentiles of the predicted concentrations in each bin from 500 simulations in the VPC.

S-Methadone

Fig 3.

Goodness of fit plots for *S*-methadone. Upper panels are the observed individual (left) and population (right) concentrations (ng/ml) versus predicted concentrations (ng/ml). Lower panels are the conditional weighted residuals (CWRES) versus the predicted concentration (ng/ml) (left) and conditional weighted residuals versus time after dose (h) (right).

Table 1

A				
	Hmong (n=76)	Non-Hmong (n=130)	P value	
Male, N (%)	54 (71.1)	72 (55.4)	< 0.05 ^A	
Age, years (SD)	56.6 (11.6)	41.7(10.7)	< 0.001 ^B	
Methadone dose, mg (SD)	54.0 (19)	82.4 (31.2)	< 0.001 ^B	
Weight, kg (SD)	61.9 (12.6)	88.9 (20.6)	< 0.001 ^B	
BMI, kg/m ² (SD)	25.7 (4.9)	30.5 (6.6)	< 0.001 ^B	
Time on methadone, yrs (SD)	6.0 (3.9)	1.9 (2.8)	< 0.001 ^B	
A χ^2 B t-test				
B				
	Caucasian (n=63)	African American (n=44)	American Indian (n=19)	Hispanic (n=4)
Male, N (%)	37 (58.7)	29 (65.9)	5 (26.3)	2 (50.0)
Age, years (SD)	39.4 (11.8)	46.5 (6.6)	39.0 (10.3)	37.3 (15.5)
Methadone dose, mg (SD)	90.7 (29.5)	66.8 (25.7)	87.2 (37.1)	99.3 (22.1)
Weight, kg (SD)	83.7 (18.4)	93.0 (20.9)	98.9 (22.6)	78.8 (18.8)
BMI, kg/m ² (SD)	29.0 (5.9)	31.2 (6.9)	34.8 (6.4)	27.2 (6.5)
Time on methadone, yrs (SD)	1.8 (2.3)	1.7 (3.0)	2.7 (3.6)	1.9 (1.7)

Table 2

Shows final parameter estimates for **R**-methadone and bootstrap analysis.

Parameter	NONMEM		Bootstrap analysis	
	Estimate (θ)	95% CI [RSE%]	Estimate (θ)	95% CI
CL/ <i>F</i> (l/h)	7.6	7.0–8.3	7.6	7.0–8.4
Hmong	0.713	0.625–0.801	0.718	0.630–0.805
(Age/48) ^{0.2}	−0.359	−0.550–0.168	−0.360	−0.565–0.149
rs2032582 GG	0.820	0.734–0.906	0.816	0.738–0.915
<i>V/F</i> (l)	311	277–345	310	279–345
IIV of CL (%CV)	34.5	[13.3]	34.4	29.7–38.9
IIV of <i>V</i> (%CV)	19.2	[78.3]	20.3	6.2–41.4
RV, proportional (%CV)	20.6	[22.6]	20.3	14.4–24.6

RSE: relative standard error; IIV: interindividual variability; RV: residual variability.

Table 3

Shows final parameter estimates for *S*-methadone and bootstrap analysis.

Parameter	NONMEM		Bootstrap analysis	
	Estimate (θ)	95% CI [RSE%]	Estimate (θ)	95% CI
CL/ <i>F</i> (l/h)	10.2	8.9–11.4	10.2	9.0–11.5
Hmong	0.676	0.592–0.760	0.679	0.600–0.770
rs2032582 GG	0.787	0.694–0.880	0.781	0.700–0.887
rs3745274 T	0.810	0.717–0.903	0.808	0.719–0.905
<i>V/F</i> (l)	224	206–253	228	207–250
IIV of CL (%CV)	39.6	[14.2]	39.1	34.0–45.0
IIV of <i>V</i> (%CV)	10.1	[161.8]	12.6	3.3–24.2
RV, proportional (%CV)	25.7	[15.1]	25.4	21.3–29.4

RSE: relative standard error; IIV: interindividual variability; RV: residual variability.