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Population Genetic-Based Pharmacokinetic Modeling of Methadone and Relationship with the QTc Interval in Opioid Dependent Patients

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Abstract

Background and objectives. Methadone is a μ-opioid agonist widly used for pain treatment and for detoxification or maintenance treatment in opioid addiction. It has been shown to exhibit large pharmacokinetic variability and concentration-QTc relationships. In this study we investigated the relative influence of genetic polymorphism and of other variables on the dose-concentration-QTc relationship.

Patients and methods. A population model for methadone enantiomers in 251 opioid dependant patients was developed using non-linear mixed effect modeling (NONMEM®). Various models were tested to characterize the pharmacokinetics of (*R)*- and (*S)*-methadone and the pharmacokinetic-pharmacodynamic relationship, while including demographics, physiological conditions, comedications and genetic variants as covariates. Model-based simulations were performed to assess the relative increase in QTc with dose upon stratification based on genetic polymorphism.

Results. A two-compartment model with first-order absorption and lag time provided the best model fit for (*R)*- and (*S)*-methadone pharmacokinetics. (*S*)-methadone clearance was influenced by CYP2B6 activity, *ABCB1 3435C>T* and α-1 acid glycoprotein level while that of (*R*)-methadone by CYP2B6 activity, *POR*28* and *CYP3A4*22*. A linear model described the methadone concentration-QTc relationship, with a mean QTc increase of 9.9ms and 19.2ms per 1000ng/ml of (*R*)- and (*S*)-methadone, respectively. Simulations with different methadone doses up to 240mg/day showed that <1% of patients would present QTc above 470ms, but might reach 6-10% in patients with a genetic status associated with a decreased methadone elimination at doses exceeding 240 mg/day.

Conclusion. Risk factor assessment, electrocardiogram monitoring, and therapeutic drug monitoring are beneficial to optimize treatment in methadone patients, especially for those who have low levels despite high methadone doses or who are at risk of overdosing.

Key points

- This study show that methadone enantionmers' concentration profiles are very variable and affect the QTc interval, which partially dependent on the patient's genetic status.
- QTc intervals predicted in this study according to patients' genetic factors confirm the need for electrocardiogram monitoring and arrhythmia risk factor assessment to improve methadone safety.
- Plasma methadone concentrations measurements by therapeutic drug monitoring may be beneficial to guide practitioners' dosing decisions, especially for those patients who have low levels despite high methadone doses or who are at risk of overdosing

Introduction

Methadone is a μ-opioid agonist used for more than 30 years for pain treatment, and for detoxification or maintenance treatment in opioid addiction. The World Health Organization has introduced this drug in the list of essential medicines in 2005 [1]. They are however numerous case reports, retrospective analyses and observational studies describing QT prolongation and torsade de pointes (TdP) in patients under methadone treatment, especially in those receiving high to very high doses of methadone [2].

Methadone is mainly administered as a chiral mixture of (*R,S*)-methadone. However the μ-opioid receptor activation is mostly due to *(R)*-methadone [3], while *(S)*-methadone is 3.5 times more potent in blocking the cardiac hERG channel, the latter being therefore at higher risk for prolonging the QT interval [4, 5]. The higher potential of the *(S)*-enantiomer to block the hERG channel was confirmed in opioid dependant patients in maintenance treatment in whom replacement of *(R,S)*-methadone by *(R)*-methadone led to a significant decrease of QT_c values[6]. However, as *(R)*-methadone is currently available in only a few countries, the prescription of *(R,S)*-methadone should in no way be limited when clinically indicated and well monitored.

The variability in methadone concentrations and effect relationship is very large. Pharmacokinetic variability has been attributed to cytochrome P450 (CYP) isoenzymes, mainly CYP3A4 and CYP2B6, and to P-glycoprotein (P-gp encoded by the ATP-binding cassette sub-family B member 1 (*ABCB1*) gene), the activities of which are genetically and environmentally determined [7]. In addition, stereoselective CYP2B6-mediated metabolism of (*S*)-methadone, as evidenced by previous in vitro/in vivo data [8-11], induces higher plasma concentrations of (*S*)- than (*R*)-methadone in CYP2B6 slow metabolizers [12, 13]. The *CYP2B6*6/*6* genotype represents about 6% of Caucasians and African-Americans, who could thus be at higher risk of developing cardiotoxicity. Cytochrome P450 reductase (POR) plays a major role in drug metabolism as all microsomal CYP receive electrons from

nicotinamide adenine dinucleotidephosphate (NADPH) through POR [14]. A large number of *POR* SNPs have now been described, which might influence CYP activities and thus drug metabolism [14]. Although stereoselective pharmacokinetics of methadone, genetic influences on *(R)*- and *(S)*- methadone concentration and concentration-effect relationships have been already explored [15-17], the relative influence of genetic polymorphism and of other variables on the dose-concentration-QTc relationship remains to be investigated.

The objectives of the study were to characterize the population pharmacokinetics of (*R)*- and (*S*)-methadone, to identify genetic and non-genetic sources of variability and to relate methadone concentrations to QTc interval in a cohort of opioid dependant patients in methadone maintenance treatment. The model served to simulate (*R*)- and (*S*)-methadone concentration-QTc interval at various dosage regimen of methadone according to the genetic status in order to quantify the expected increase of QTc in the different groups.

Materials and methods

Study population

Plasma *(R)*- and *(S)*-methadone levels, measured by liquid chromatography coupled with mass spectroscopy [18], and QTc measurements were obtained from 244 opioid-dependent patients in 5 methadone-dispensing centers in Geneva, Lausanne, Bern, and Montreux, Switzerland [7]. Steady-state concentration samples with matched QTc interval determinations (Fridericia-corrected) were collected around peak (4h) and/or trough (24h) drug levels. In addition, seven opioid-dependent patients taking part in an interaction study [19] provided 11 *(R)*- and *(S)*-methadone concentrations per patient, measured by liquid chromatography coupled with ultraviolet detection [20] , collected between 0.5 and 23 h post dose under steady-state conditions. Mean (± SD) methadone dose was 123 (± 75) mg (range 3-400 mg) and was given once or twice daily. The ethics committees of the corresponding centers approved the project and all participants gave written informed consent for genetic testing.

Analytical method

Genotyping

Genomic DNA was extracted from EDTA blood samples with the FlexiGene DNA Kit (Qiagen, Hombrechtikon, Switzerland). All the SNPs, with the exception of *CYP2D6*5*, *CYP2D6*xN* and *CYP3A4*22*, were detected by real-time polymerase chain reaction (PCR) with the use of 5'-nuclease allelic discrimination assays (ABI PRISM 7000 Sequence Detection System, Applied Biosystems, Rotkreuz, Switzerland) with primers and probes obtained from Applied Biosystems. The tested genetic polymorphisms are listed in **Table 2** and were analyzed as previously described except for *CYP3A4*22* [7, 21-23]. *CYP3A4*22* was genotyped with a custom Illumina iSelect genotyping array on an iScan equipped platform (Illumina, San Diego, California). Quality control was assessed by the call rate (>96%), GC score (>0.15) and matched gender. Genetic data was called by using GenomeStudio software (version 2011.1, Illumina, San Diego, California).

Model-based pharmacokinetic modeling

The model building process consisted of: (i) a population pharmacokinetic model for *(R)*- and *(S)*-methadone using rich sampling data; (ii) an extension to the whole dataset while taking into account potential influencing variables; (iii) a simultaneous pharmacokinetic-pharmacodynamic (PK-PD) analysis for the concentration-QTc interval relationship evaluation.

Structural models

A stepwise procedure was used to find the models that fitted methadone data at best. The final pharmacokinetic model was a two-compartment model with first-order absorption and lag time for both drugs. The estimated parameters are clearance (CL), volume of distribution of the central compartment (Vc), volume of distribution of the peripheral compartment (Vp), intercompartmental clearance (Q), absorption rate constant (ka) and lag-time (Tlag). Since

methadone was only administered orally, CL, Vc, Vp and Q represent apparent values. Methadone was given as a racemic mixture composed of 50:50 of both enantiomers, and methadone dose was reduced by half by fixing the relative bioavailability to 0.5 for the separate analysis of each moiety. Exponential errors following a lognormal distribution with mean zero and variance ω^2 were assumed for the description of the interindividual variability. A proportional error model was used to model the residual variability with mean zero and variance Σ^2. Methadone elimination half-lives ($t_{½\alpha}$ and $t_{½\lambda}$), volume of distribution at steady-state (Vd_{ss}) and mean absorption time (MAT= 1/ka + lag-time) were derived using classical equations.

Covariate models

The available covariates were demographics (sex, body weight, race), physiopathological (α1-glycoprotein, albumin), environmental (smoking, co-medications) (**Table 1**) and tested genetic polymorphisms (**Table 2**). The potential influence of available covariates were explored graphically and then tested for significance into the model. The typical value of a parameter was modeled to depend on a covariate (continuous variables centered on the mean; categorical covariates being coded as indicator variables 0 or 1), testing linear and non-linear relationships, as appropriate. Comedications were classified into inducers or inhibitors of CYP3A4 and CYP2B6 and inhibitors of CYP2D6 [24-27] (Table 1). Comedications potentially affecting the QT intervals were categorized as drugs with a known, possible or conditional risk of torsade de pointes according to CredibleMeds.org [28] and patients were grouped according to their highest risk comedication. Genetic variants were classified into 3 groups: reference allele (Ref), heterozygote (Het) loss/decrease/gain of function (LOF/DOF/GOF) and homozygote (Hom) LOF/DOF/GOF variants. LOF and DOF alleles will be defined as DOF thereafter. An activity score for *CYP2B6* was derived to test for the effect of a combination of *CYP2B6*6, *5* and **11* alleles. In this model, a score of 2 was attributed to fully functional alleles *CYP2B6*1/1, *5/*5, *1/*4, *1/*5, *4/*5,* a score of 1 to *CYP2B6*1/*11, *1/*6, *4/*6, *5/*6* and a score of 0 to *CYP2B6*6/*6* and **6/*11*. The

relationship between methadone CL and the number of functional alleles was first tested using a rich model (eq.1) and competing models that accounted for gene effect as a function of the number of functional alleles using linear (eq.2), power (eq.3) and square root (eq.4) relationships with either additive or proportional (not shown) effect, as follows:

$$CL = CL_0 \cdot (1+\theta_1 I_1) \cdot (1+\theta_2 I_2) \quad (1)$$

$$CL = CL_0 + \theta_1 \cdot n \quad (2)$$

$$CL = CL_0 + \theta_1^{\,n} \quad (3)$$

$$CL = CL_0 + \theta_1 \sqrt{n} \quad (4)$$

where n =1, 2 represents the functional score and θ_1 the average contribution per active allele above the clearance level of Hom DOF individuals (CL_0). The joint influence of functional alleles on methadone CL was first tested using pair-wise conjunction of *CYP2B6* with the other *CYP* alleles, to finally build up the model including all influent genetic variants. The impact of functional alleles on methadone CL was further explored using the dominant (Hom/Het DOF vs. Ref alleles) and recessive (Hom DOF vs. Het DOF/Ref alleles) models.

The investigation of the joint influence of *CYP2B6* and *ABCB1 3435C>T* alleles is shown as an example. The richest model was:

$$CL = CL_0 + \theta_{01} I_{01} + \theta_{02} I_{02} + \theta_{10} I_{10} + \theta_{11} I_{11} + \theta_{12} I_{12} + \theta_{20} I_{20} + \theta_{21} I_{21} + \theta_{22} I_{22} \quad (5)$$

where here CL_0 is CL in individuals Hom DOF for both genes and I_{ij} is an indicator variable that takes the value of 1 if the individual carries the *CYP2B6* ith genotype and the *ABCB1 3435C>T* jth genotype and is "0" otherwise, and each θ_{ij} estimate the absolute change in CL among the different genotypic groups. The following competing models were evaluated:

$$CL = CL_0 + \theta_1 \cdot \sqrt{p} + (\theta_2 I_1 + \theta_3 I_2) \quad (6)$$

$$CL = CL_0 + \theta_1 \cdot \sqrt{p} + (\theta_2 \cdot n) \quad (7)$$

$$CL = CL_0 + \theta_1 \cdot \sqrt{p} + \theta_2 \cdot \sqrt{q} \quad (8)$$

where *p* indicates the functional score for *CYP2B6* and *q* the score for *ABCB1 3435C>T*. In eq.6, the contribution of *ABCB1 3435C>T* (θ_2, θ_3) is investigated using all genetic groups on *CYP2B6* polymorphism and in reduced models and testing linear (eq.7) and square root functions (eq.8) for both genes.

Concentration-QTc modeling

The relationship between methadone concentrations and the Fridericia heart-rate corrected QT_c [29] interval were explored using linear and log-linear models of the general form:

$$QTc_{ij} = Intercept_i + Slope_i \cdot Conc_{ij} + \varepsilon_{ij} \quad (9)$$

where $Slope_i$ and $Intercept_i$ are the slope and the intercept for the i^{th} individual and QTc_{ij} and is the Fridericia's corrected QT interval and $Conc_{ij}$ methadone concentration for the i^{th} individual and the j^{th} time point. The residual variability, ε_{ij}, was included assuming an additive error with mean zero and variance Σ^2 and an interindividual variability on the slope and intercept was tested assuming a normal distribution with mean zero and variance ω^2. Available potentially influencing factors on the QTc interval were sex, potassium and calcium levels, comedications affecting the QTc interval and were included in the model using linear functions.

Parameter Estimation and Selection

NONMEM® [30](version 7.2, NM-TRAN, version II) was used with the FOCE INTERACTION method to fit the data. The minimum objective function value (ΔOF) provided by NONMEM®, (-2 log likelihood, approximate χ^2 distribution) was used to discriminate between hierarchical models using the log likelihood test. A model was considered superior to another nested model when the OFV value was reduced by at least 3.84 points ($p < 0.05$). Covariate analysis comprised forward selection of influential factors followed by backward deletion and were

retained in the final model at the statistical level of $p < 0.01$. The most appropriate model for the concentration-QTc relationship was selected based on Akaike information criteria and the log likelihood test for the influence of covariates. Model assessment was based on diagnostic plots along with the measure of the standard errors, the correlation matrix of parameter estimates, the size of residual errors and eta-shrinkage.

Model validation

The stability and the performance of the final population pharmacokinetic model were validated by the bootstrap method using 2000 bootstrap resampling with replacement (Perl-speaks-NONMEM version 3.2.4 (http://psn.sourceforge.net/)). The median and the 95% confidence interval of each parameter obtained with the bootstrapped data were compared to the parameters of original dataset. In addition, simulations were performed in 1000 to predict the 2.5^{th}, 50^{th} and 97.5^{th} percentiles of the concentrations and QTc intervals and compared with observed data. The figures were generated using GraphPad Prism (Version 4.00 for Windows, GraphPad Software, San Diego California USA, (www.graphpad.com).

Simulations

Simulations for *(R)- and (S)*-methadone plasma and QT interval were performed in 1000 individuals for doses ranging from 80 mg to 280 mg once daily at steady-state. The volume of distribution of (*S)*-methadone was fixed for a typical patient with a median AAG level of 0.97 g/L. A “lowest” CL and “best” CL value were calculated for *(R)*- and *(S)*-methadone stratified according to the genetic status. Average QT interval predictions with the 95% prediction intervals ($PI_{95\%}$) for each dosage regimen were retrieved. In addition, the percentage of patients with absolute QT above the thresholds of >450 ms and > 470 ms associated with an increased risk of cardiotoxicity was derived.

Prediction of *(R,S)*-methadone plasma concentration threshold for QTc prolongation risk

Receiver operating characteristic (ROC) analyses and graph were performed using GraphPad Prism (Version 6.05 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com) to predict a threshold of *(R,S)*-methadone plasma concentration for increased risk of QTc above 450 ms.

Results

A total of 504 *(R)*- and *(S)*-methadone plasma concentrations and 317 QTc intervals were used for the analysis. A summary of the population characteristics and of the tested genetic polymorphisms are presented in **Tables 1 and 2**.

Population Pharmacokinetic Analysis

Structural model for *(R)*- and *(S)*-methadone. Analyses from 7 individuals with rich sampling showed that a two-compartment model with first order absorption fitted the data better than a one-compartment model for both enantionmers ($\Delta OF>-23;p<0.001$); the addition of a lag time significantly improved the fit for both compounds ($\Delta OF>-34;p<0.001$). In addition to CL, assignment of an interindividual variability on Ka improved the fit for *(R)*- ($\Delta OF=-43;p<0.001$) and *(S)*-methadone ($\Delta OF=-37;p<0.001$), as well as on Q for the latter ($\Delta OF=-7;p<0.01$). This model adequately fitted *(R)*- and *(S)*-methadone concentrations of the whole population. The lack of drug level measurements during the absorption phase prevented from a precise estimation of Ka; this parameter and the lag time were thus fixed to the estimates from the rich data analysis. Addition of an interindividual variability on Vc and Q ($\Delta OF>-34;p<0.001$) improved the description of both enantiomers' data, with a correlation between CL and Vc ($\Delta OF>-12;p<0.01$). Allowing Ka and the peripheral volume of distribution (Vp) to vary did not improve the model fit ($\Delta OF>-1.2$).

Covariate modeling on *(S)*-methadone. Among the non-genetic covariates, only α-1 acid glycoprotein (AAG) concentrations influenced Vc ($\Delta OF=-12.8;p<0.001$), indicating a 25% increase in volume upon AAG doubling.

Univariate analyses testing the influence of genetic polymorphisms revealed that *CYP2B6*6* had a marked impact on (*S*)-methadone elimination (ΔOF=-14.0,p<0.001), with an estimated CL of 10.6 l/h, 9 l/h and 6.6 l/h in Ref, Het decrease of function allele (DOF) and Hom DOF patients, respectively. A trend for an influence of the *CYP2B6 rs2279344* polymorphism was found (ΔOF=-3.8,p=0.051), suggesting a 14% decrease in *(S)*-methadone CL in Hom DOF carriers compared to the other groups. *CYP2B6*11* (ΔOF=-2.4;p=0.12), and *CYP2B6*5* (ΔOF=-0.7;p=0.4) did not show any influence. The use of an activity score combining *CYP2B6*6, *5 and *11* improved the fit (ΔOF=-20.4;p<0.001), providing very close estimates of CL to the model including *CYP2B6*6* solely, and was retained for further analyses. The relationship between CL and the number of *CYP2B6* functional alleles could adequately be described by a square root model (eq.4). This model estimated a CL of 6.2 l/h in Hom DOF patients that increased by $3.1 \cdot \sqrt{n}$ (n=number of functional allele). Polymorphism in *POR*28* showed a significant influence on CL (ΔOF=-4.1;p<0.05); a 20% increase in *(S)*-methadone CL in Hom gain of function allele (GOF) (*POR*28 TT*) compared to Ref and Het GOF patients was observed. *CYP3A4*22* Het DOF was associated with a non-significant 15% decrease in *(S)*-methadone CL (ΔOF=-3,p=0.08). Variations in the *ABCB1 3435C>T* (ΔOF=-5.9;p<0.05), *2677G>T/A* (ΔOF=-7;p<0.01), *61A>G* (ΔOF=-4.5;p<0.05) and *1199G>A* (ΔOF=-9.2;p<0.01) significantly affected *(S)*-methadone CL. Patients carrying two variant alleles of these polymorphisms had a 15 to 30% higher methadone elimination compared to Het DOF and Ref patients. *CYP3A5*3* did not show any influence on *(S)*-methadone CL (ΔOF=-2.4;p=0.12). No other genetic polymorphism showed any influence.

Stepwise inclusion of the significant genetic variants in the multivariate analyses identified *CYP2B6* activity score and *ABCB1 3435C>T* polymorphism as significant covariates on CL. Both allele influences were best captured using a square root model for *CYP2B6* functional alleles with an additional influence of *ABCB1 3435C>T* for Hom DOF carriers of this gene (eq. 6). No statistical difference in CL between *ABCB1 3435C>T* Het DOF and Hom DOF stratified for *CYP2B6* was observed, and both linear (eq. 7) and square root models (eq.8) described the data as well (ΔOF< -0.7). All other significant allelic variants were correlated to

both genes and did not remain statistically significant. The influence of AAG levels on Vc was retained in the final model. This final model explained 4%, 16% and 7% of the interindividual variability on CL, Vc and Q, respectively.

Covariate modeling on *(R)*-methadone. No non-genetic factors showed any influence on *(R)*-methadone disposition. Inclusion of *CYP2B6*6* (ΔOF=-7.0;p<0.01) and *CYP2B6* activity score (ΔOF=-7.5;p<0.01) improved the description of *(R)*-methadone elimination. These models estimated a CL of 10.4 l/h, 9.5 l/h and 8.0 l/h in Ref, Het DOF and Hom DOF patients, respectively. The use of a square root model according to the number of functional alleles fitted the data as well as the rich model, with a CL of 7.9 l/h in Hom DOF that increased by $1.7 \cdot \sqrt{n}$. A significant influence of the *CYP2B6 rs8192719* polymorphism was found (ΔOF=-7.9; p<0.01), but not of the *CYP2B6 rs2279344* (ΔOF=-3.5;p=0.061). Variations in the *POR*28* (ΔOF=-7.1;p<0.01) and *CYP3A4*22* (ΔOF=-5.0;p<0.05) influenced the model fit, revealing an increase in CL by 14% in Het or Hom GOF carriers of *POR*28* and a decrease of 22% in Het DOF *CYP3A4*22* compared to the Ref groups. *CYP3A5*3* did not show any influence on *(R)*-methadone CL (ΔOF=0). No other genetic polymorphism showed any influence.

The *CYP2B6* activity score, *POR*28* and *CYP3A4*22* remained statistically significant in the multivariate analysis. *CYP2B6 rs8192719* was not retained due to a strong linkage with *CYP2B6*6.* The joint effect of the three alleles was best fitted using an additive model (eq. 6), with no further improvement while using competing models (eq. 7 or eq. 8). This final model explained 5%, 6% and 16% of the interindividual variability on CL, Vc and Q, respectively.

Methadone concentration-QTc interval Analysis

The concentration-QT_c interval relationship was fitted using a linear model, with no difference observed using a log-linear model (ΔOF<-1.83). The assignment of an interindividual variability on the intercept parameter improved significantly the fit (ΔOF=-62 for *(R)*- and

ΔOF=-64 for *(S)*-Methadone;p<0.001), but no variability on the slope parameter was found (ΔOF=0). A slightly non-significant higher intercept QTc level of about 3 ms was observed in women compared to men and no difference in slope was observed (ΔOF>-2). Neither potassium, nor calcium levels significantly influenced the concentration-QTc relationship (ΔOF>-1.3), nor did comedications at risk of QTc prolongation. The final population parameters are presented in **Table 3**. Goodness-of-fit plots are presented in **Supplementary Figure 1**.

Model Validation

The median parameter estimates obtained with bootstrap with the 95% confidence interval (CI) are presented in **Table 3**. The parameter estimates of the final population pharmacokinetic model lied within the 95% CI of the bootstrap results suggesting that the model was acceptable. The visual predictive check of the observed methadone concentrations versus time and the concentration-QTc plots is shown in **Figure 1**.

Simulations

Simulations of (*R*)- and (*S*)-methadone concentrations for 80 mg to 320 mg once daily assuming a “best” and “lowest” elimination of each enantiomer according to influencing genetic effects are shown in **Figure 2**. The probability of the QT_C value being above the 450 ms threshold is increased by 1.5 to 2 times depending on the genotypic status. Less than 4% of patients would present QTc above 450 ms at the dose levels below or equal to 160 mg daily, but this number might increase up to 10% for patients at doses exceeding 240 mg/day with a genetic status associated with a decreased methadone elimination, particularly observed for *(S)*-methadone. Less than 1% of patients would present QTc values above 470 ms according to our model.

Prediction of *(R,S)*-methadone plasma concentration threshold for QTc prolongation risk

The ROC analysis indicates that a plasma concentration of *(R,S)*-methadone above 656 ng/ml is the best predictor for a QTc above 450 ms (**Supplementary Figure 2;** sensitivity=79%; specificity=68%; ROC AUC=0.69 $\pm$ 0.06; 95%CI=0.57-0.81, p=0.01).

Discussion

We developed a population pharmacokinetic-pharmacodynamic model for methadone in a cohort of opioid patients, and could quantify the relative contribution of genetic and non-genetic factors affecting drug disposition, its consequences on the QTc interval and predict QTc increase according to dose and patients' genetic status.

Estimates of (*R*)- and (*S*)-methadone pharmacokinetic parameters are in good accordance with previous results [16, 17]. No demographic covariates showed any influence on these compounds' kinetics, except for an influence of AAG on the volume of distribution of (*S*)-methadone. The lack of influence of protein binding on (*R*)-methadone could be related to a lower protein binding of this enantiomer [31]. As expected, a marked interindividual variability in methadone pharmacokinetics was observed, even more pronounced for (*S*)-methadone. This large variability is typical of drugs that are metabolized by polymorphic enzymes such as CYP3A4 and CYP2B6. Few strongly interacting co-medications were taken by patients, thus reducing the power to detect any influence of comedications. Since CYP2D6 seems only moderately involved in methadone metabolic pathway [7, 32] an influence of CYP2D6 inhibitors was not expected.

A genetic influence of *CYP2B6*6* was observed on both enantiomers, with a more important effect on (*S*)-methadone. Its clearance is reduced by 37% in *CYP2B6* Hom DOF carriers, whereas (*R*)-methadone clearance is decreased by 21%. These data confirm the

stereoselectivity of the CYP2B6 isoform for (*S*)-methadone, previously reported [8, 10-13, 17, 33]. The lack of influence of *CYP2B6*11* could be explained by its low minor allele frequency (0.8%). *CYP2B6*5* was also not found to influence methadone elimination in agreement with an *in vitro* moderately decreased activity [33], despite the recent finding of its overrepresentation in a group of patients with low methadone concentration (thus suggesting an increased CYP2B6 activity) [22]. We therefore defined the CYP2B6 activity score by combining *CYP2B6*6*, **11 and *5*, the major known DOF and loss of function (LOF) alleles of *CYP2B6* in Caucasians.

We report in the present study for the first time a decrease in methadone clearance in carriers of the *CYP3A4*22* allele. This variant was previously shown to affect CYP3A4 mRNA expression and enzyme activity, the mutant carriers presenting approximately 2.5-fold lower CYP3A4 activity [34], which was verified by CYP3A-phenotyping probes midazolam and erythromycin [35]. In univariate models, the *CYP3A4*22* allele significantly decreased *(R)*-methadone clearance (-23%) and non-significantly *(S)*-methadone clearance (-15%). This small difference is most likely due to the stronger influence of CYP2B6 on *(S)*-methadone rather than a real stereoselectivity of CYP3A4. The lack of CYP3A4 stereoselective metabolism of methadone has been previously shown *in vivo* [7] and *in vitro* [8, 10, 11]. On the other hand, *CYP3A5*3* did not seem to influence either *(S)*- or *(R)*-methadone elimination, in accordance with *in vitro* [9] and *in vivo* results [7].

An increase in both enantiomers' elimination in *POR*28* Het or Hom carriers was observed in univariate analyses. This is in accordance with the increased CYP3A activity observed by midazolam phenotyping in *POR*28* Hom GOF carriers [21, 36]. Conflicting results were also found [37, 38], which might suggest substrate specific-effects of POR variants on CYP3A activities [14]. In multivariate analyses, the influence of the *POR*28* polymorphism was only observed for *(R)*-methadone, possibly due to the more important contribution of CYP2B6 to *(S)*-methadone thus masking the POR influence.

Methadone is a substrate and inhibitor of the PgP coded by the *ABCB1* gene. We observed a small reduction in (*S*)-methadone clearance in carriers of one or two DOF alleles of the gene, whereas no effect was found for (*R*)-methadone. These results are in agreement with an *in vitro* experiment suggesting a weak stereoselectivity for the transport of the *(S)*-enantiomer [39]. Moreover, a recent population pharmacokinetic study also found an association of *ABCB1 2677G>T/A* with a 20% reduction of the clearance of (*R*)- and (*S*)-methadone, but did not observe any stereoselectivity [17].

The concentration-QTc relationship indicate that mean QTc is increased by 9.9 ms and 19.2 ms per 1'000 ng/ml of (*R*)- and (*S*)-methadone, respectively, in line with previously reported values of 17 ms for the racemate ($CI_{90\%}$ 12-22) [15]. The magnitude of the effect is small and of modest clinical relevance at low methadone dosage. The range of QTc interval measured in our population was slightly higher than in the Framingham study [40] but did not include extreme QTc values, which might explain the modest concentration-effect relationship. Our results however suggest a more pronounced QTc increase with *(S)*-methadone, and confirm the more potent inhibition of the hERG channel by this enantiomer [4]. Considering that both enantiomers' concentrations can increase in patients with a genetic-related poor elimination profile and the confirmed link between exposure and the risk of QTc prolongation, the need for caution is reinforced, especially at higher methadone dosage. This need for caution has been confirmed by recent studies on drug-induced QTc prolongation. A large study on the prevalence of drug-induced long QT in adult psychiatric patients reported that prolonged QTc was significantly associated with methadone, which was the most frequent drug among patients with drug-induced long QT [41]. Furthermore, among the reported 12 cases of sudden death and/or torsade de pointes (TdP), seven patients had received methadone. In another study aimed to determine the associated factors for prolonged QTc and the development of TdP, methadone was by far the leading medication implicated in the development of TdP and an independent predictor in both univariate and multivariate analyses despite the fact that it was not the most common QT-prolonging medication in their

population [42].

One limitation of our study is that the relationships between both enantiomers and QTc could only be described with a linear relationship. QTc measurements were performed at two times post-drug intake, which prevented from the possibility to characterize the full pharmacokinetic-pharmacodynamic relationship and the relative increase in QTc interval after dose administration. Sex, calcium and potassium levels did not influence QTc in this study, neither did co-adminstration of drugs at risk for QT prolongation. Potassium and calcium levels were in the normal range, which might have limited to detect any association. Only few drugs with known risk of torsade de pointes were prescribed in this population and the influence of these drugs is expected to be modest within the normal range of QTc interval. Other patient's risk factor, such as the use of illicit hERG blockers were not accounted for in our model, which is another study limitation.

As previously reported [15], a simulation approach allowed to describe the concentration- QT interval relationship, while integrating for the first time genetic influence on methadone elimination. Patients carrying mutation of the *CYP2B6*6* are at risk of exhibiting high methadone concentrations, in particular *(S)*-methadone, and QTc prolongation above the 450 ms threshold. This risk is even more pronounced in case of additional variation such as *CYP3A4*22* and *ABCB1 3435C>T* affecting *(R)*- and *(S)*-methadone elimination, respectively. Variation in the *POR*28* would only partly compensate for a CYP2B6-associated reduced *(R)*-methadone elimination.

The ROC analysis predicted a threshold of 656 ng/ml *(R,S)*-methadone plasma concentration for QTc prolongation above 450 ms. This value should be considered cautiously as only 14 values of QTc (among 317) were above 450 ms but is close to the clinically determined threshold of 800 ng/ml previously proposed [31].

Despite an important influence of CYP2B6 polymorphisms and other genes, the major part of methadone pharmacokinetic variability remains unexplained. The very large variability in plasma methadone concentrations renders the dose-concentration relationship difficult to predict. Plasma methadone concentrations measurements by therapeutic drug monitoring may be beneficial to guide practitioners' dosing decisions, especially for those patients who have low levels despite high methadone doses or who are at risk of overdosing (i.e poor metabolizer status, high methadone dose or suspicion of a drug-drug interaction). As previously reported, trough plasma concentrations of 250 ng/ml for *(R)*-methadone or 400ng/ml for *(R,S)*-methadone might be used as target values in cases of nonresponse [31]. Due to the widely unexplained pharmacokinetic variability and the fact that pharmacodynamic pathway–related genes in the heart and other factors such as electrolyte disturbances may also be important, our data confirm the need for electrocardiogram monitoring and arrhythmia risk factor assessment as determined in clinical practice for patients receiving doses above 100 or 120 mg/day [43, 44] or for unusual situation with trough *(R,S)*-methadone plasma concentrations above 660-800 ng/ml [31].

Compliance with ethical standards

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Conflict of interest : CBE has received research support from Takeda and from the Roche Organ Transplantation Research Foundation in the past 3 years. He has received honoraria for conferences or teaching CME courses from, Astra Zeneca, Janssen-Cilag, Lundbeck, Merck Sharp & Dohme, Mepha, Otsuka, Sandoz, Servier, and Vifor-Pharma in the past 3 years.

CC, SC, MG declare that they have no conflict of interest that might be relevant to the contents of this manuscript."

Legend to figures

Figure 1 : (*R*)- and (*S*)-methadone plasma concentration-time profile (stratified for a 100 mg-daily steady-state dose)(upper panels); QTc vs. time (middel panel) and observed concentration vs. QTc (lower panels), with population prediction (solid line) and 95% prediction interval (dashed lines).

Figure 2 : Model-based predicted percentage of patients with QT_C value above the threshold value of 450 ms (solid lines) and 470 ms (dashed lines) for different methadone daily doses and stratified according to the genetic effect for **(A)** (*R*)-methadone and **(B)** (*S*)-methadone. Black symbols: « best » *(R)*- or *(S)*-methadone clearance and white symbols: «lowest» *(R)*- or *(S)*-methadone clearance based on the final model estimates. For (*S*)-methadone, the "lowest" CL value is a *CYP2B6* activity score of 0 (decrease of function allele; DOF) and *ABCB1 3435 CC or CT* (CL= 6.14 L/h) and the « best » CL value is a *CYP2B6* activity score of 2 (reference allele; Ref) and *ABCB1 3435 TT* (CL=11.65 L/h). For (*R*)-Methadone, the "lowest" CL is a *CYP2B6* activity score of 0 (DOF), *POR*28* (homozygote gain of function allele; GOF) and *CYP3A4*22* heterozygote DOF (CL=7.53 L/h) and the "best" CL is a *CYP2B6* activity score of 2 (Ref), *POR*28* Ref and *CYP3A4*22* Ref (CL=10.97 L/h).

Supplementary Figure 1: Goodness-of-fit plots for **A :** (*R*)-methadone and **B :** (*S*)-methadone population and individual pharmacokinetic and QTc predictions vs observations and conditional weighted residuals (CWRES) vs. time after last dose.

Supplementary Figure 2 : Receiver Operating Characteristic (ROC) curve indicating the best *(R,S)*-methadone plasma concentration threshold to predict a QTc prolongation above 450 ms. **(R,S)*-methadone plasma concentration value; sensitivity and specificity enclosed in parentheses.

References

1. World Health Organization. WHO model list of essential medicines 2005. http://www.who.int/medicines/publications/essentialmedicines/en/index.html; Accessed 15 Mar 2006.

2. Chou R, Weimer MB, Dana T. Methadone overdose and cardiac arrhythmia potential: findings from a review of the evidence for an American Pain Society and College on Problems of Drug Dependence clinical practice guideline. J Pain. 2014;15(4):338-65.

3. Kristensen K, Christensen CB, Christrup LL. The mu1, mu2, delta, kappa opioid receptor binding profiles of methadone stereoisomers and morphine. Life Sci. 1995;56:L45-L50.

4. Eap CB, Crettol S, Rougier JS, Schlapfer J, Sintra GL, Deglon JJ, et al. Stereoselective block of hERG channel by (S)-methadone and QT interval prolongation in CYP2B6 slow metabolizers. Clin Pharmacol Ther. 2007;81(5):719-28.

5. Lin C, Somberg T, Molnar J, Somberg J. The Effects of Chiral Isolates of Methadone on the Cardiac Potassium Channel IKr. Cardiology. 2009;113(1):59-65.

6. Ansermot N, Albayrak O, Schlapfer J, Crettol S, Croquette-Krokar M, Bourquin M, et al. Substitution of (R,S)-methadone by (R)-methadone: impact on QTc interval. Arch Intern Med. 2010;170(6):529-36.

7. Crettol S, Déglon JJ, Besson J, Croquette-Krokkar M, Hämmig R, Gothuey I, et al. ABCB1 and cytochrome P450 genotypes and phenotypes: influence on methadone plasma levels and response to treatment. Clin Pharmacol Ther. 2006;80(6):668-81.

8. Gerber JG, Rhodes RJ, Gal J. Stereoselective metabolism of methadone N-demethylation by cytochrome P4502B6 and 2C19. Chirality. 2004;16(1):36-44.

9. Kharasch ED, Hoffer C, Whittington D, Sheffels P. Role of hepatic and intestinal cytochrome P450 3A and 2B6 in the metabolism, disposition, and miotic effects of methadone. Clin Pharmacol Ther. 2004;76(3):250-69.

10. Totah RA, Sheffels P, Roberts T, Whittington D, Thummel K, Kharasch ED. Role of CYP2B6 in stereoselective human methadone metabolism. Anesthesiology. 2008;108(3):363-74.

11. Chang Y, Fang WB, Lin SN, Moody DE. Stereo-Selective Metabolism of Methadone by Human Liver Microsomes and cDNA-Expressed Cytochrome P450s: A Reconciliation. Basic Clin Pharmacol Toxicol. 2011;108(1):55-62.

12. Crettol S, Déglon JJ, Besson J, Croquette-Krokkar M, Gothuey I, Hämmig R, et al. Methadone enantiomer plasma levels, CYP2B6, CYP2C19 and CYP2C9 genotypes, and response to treatment. Clin Pharmacol Ther. 2005;78:593-604.

13. Wang SC, Ho IK, Tsou HH, Tian JN, Hsiao CF, Chen CH, et al. CYP2B6 polymorphisms influence the plasma concentration and clearance of the methadone S-enantiomer. J Clin Psychopharmacol. 2011;31(4):463-9.

14. Pandey AV, Sproll P. Pharmacogenomics of human P450 oxidoreductase. Front Pharmacol. 2014;5:103.

15. Florian J, Garnett CE, Nallani SC, Rappaport BA, Throckmorton DC. A modeling and simulation approach to characterize methadone QT prolongation using pooled data from five clinical trials in MMT patients. Clin Pharmacol Ther. 2012;91(4):666-72.

16. Foster DJR, Somogyi AA, White JM, Bochner F. Population pharmacokinetics of (R)-, (S)- and rac-methadone in methadone maintenance patients. Br J Clin Pharmacol. 2004;57(6):742-55.

17. Bart G, Lenz S, Straka RJ, Brundage RC. Ethnic and genetic factors in methadone pharmacokinetics: a population pharmacokinetic study. Drug Alcohol Depend. 2014;145:185-93.

18. Ansermot N, Rudaz S, Brawand-Amey M, Fleury-Souverain S, Veuthey JL, Eap CB. Validation and long-term evaluation of a modified on-line chiral analytical method for therapeutic drug monitoring of (R, S)-methadone in clinical samples. J Chromatogr B. 2009;877(23):2301-7.

19. Benmebarek M, Devaud C, Gex-Fabry M, Powell Golay K, Brogli C, Baumann P, et al. Effects of grapefruit juice on the pharmacokinetics of the enantiomers of methadone. Clin Pharmacol Ther. 2004;76:55-63.

20. Eap CB, Finkbeiner T, Gastpar M, Scherbaum N, Powell K, Baumann P. Replacement of (R)-methadone by a double dose of (R,S)-methadone in addicts: interindividual variability of the (R)/(S) ratios and evidence of adaptive changes in methadone pharmacokinetics. Eur J Clin Pharmacol. 1996;50:385-9.

21. Oneda B, Crettol S, Jaquenoud Sirot E, Bochud M, Ansermot N, Eap CB. The P450 oxidoreductase is associated with CYP3A activity in vivo as measured by the midazolam phenotyping test. Pharmacogen Genomics. 2009;19(11):877-83.

22. Dobrinas M, Crettol S, oneda B, Lahyani R, Rotger M, Choong E, et al. Contribution of CYP2B6 alleles in explaining extreme (S)-methadone plasma levels: a CYP2B6 gene resequencing study. . Pharmacogen Genomics. 2013;23(2):84-93.

23. Crettol S, Venetz JP, Fontana M, Aubert JD, Ansermot N, Fathi M, et al. Influence of ABCB1 genetic polymorphism on cyclosporine intracellular concentrations in transplant recipients. Pharmacogen Genomics. 2008;18:307-15.

24. Service de pharmacologie et toxicologie clinique; Hôpitaux Universitaires Genève. CYP P450 Drug Interactions 2011; http://pharmacoclin.hug-ge.ch/_library/pdf/cytp450.pdf. Accessed 13 Dec 2011.

25. Division of Clinical Pharmacology, Indiana University Department of Medicine. Cytochromes P450 drug-interaction table 2009. http://medicine.iupui.edu/clinpharm/ddis/table.aspx. Accessed 9 Oct 2012.

26. Compendium Suisse des Medicaments 2014. http://www.swissmedicinfo.ch. Accessed 24 Feb 2014.

27. Walsky RL, Astuccio AV, Obach RS. Evaluation of 227 Drugs for In Vitro Inhibition of Cytochrome P450 2B6. J Clin Pharmacol. 2006;46(12):1426-38.

28. Woosley RL, Romero KA. QT drugs List 2015. www.Crediblemeds.org. Accessed 10 Aug 2015.

29. Fridericia LS. The duration of systole in an electrocardiogram in normal humans and in patients with heart disease. 1920. Ann Noninvasive Electrocardiol. 2003;8(4):343-51.

30. NONMEM users guides (1989-2006). Maryland, USA: Icon Development Solutions, Ellicott City; 2007.

31. Eap CB, Buclin T, Baumann P. Interindividual variability of the clinical pharmacokinetics of methadone: Implications for the treatment of opioid dependence. Clin Pharmacokinet. 2002;41(14):1153-93.

32. Begré S, von Bardeleben U, Ladewig D, Jaquet-Rochat S, Cosendai-Savary L, Powell Golay K, et al. Paroxetine increases steady-state concentrations of (R)-methadone in CYP2D6 extensive but not poor metabolizers. J Clin Psychopharmacol. 2002;22(2):211-5.

33. Gadel S, Friedel C, Kharasch ED. Differences in Methadone Metabolism by CYP2B6 Variants. Drug Metab Dispos. 2015;43(7):994-1001.

34. Wang D, Guo Y, Wrighton SA, Cooke GE, Sadee W. Intronic polymorphism in CYP3A4 affects hepatic expression and response to statin drugs. Pharmacogenomics J. 2011;11(4):274-86.

35. Elens L, Nieuweboer A, Clarke SJ, Charles KA, de Graan AJ, Haufroid V, et al. CYP3A4 intron 6 C>T SNP (CYP3A4*22) encodes lower CYP3A4 activity in cancer patients, as measured with probes midazolam and erythromycin. Pharmacogenomics. 2013;14(2):137-49.

36. Yang G, Fu Z, Chen X, Yuan H, Yang H, Huang Y, et al. Effects of the CYP oxidoreductase Ala503Val polymorphism on CYP3A activity in vivo: a randomized, open-label, crossover study in healthy Chinese men. Clin Ther. 2011;33(12):2060-70.

37. Elens L, Nieuweboer AJ, Clarke SJ, Charles KA, de Graan AJ, Haufroid V, et al. Impact of POR*28 on the clinical pharmacokinetics of CYP3A phenotyping probes midazolam and erythromycin. Pharmacogenet Genomics. 2013;23(3):148-55.

38. Agrawal V, Choi JH, Giacomini KM, Miller WL. Substrate-specific modulation of CYP3A4 activity by genetic variants of cytochrome P450 oxidoreductase. Pharmacogenet Genomics. 2010;20(10):611-8.

39. Crettol S, Digon P, Golay KP, Brawand M, Eap CB. In vitro P-glycoprotein-mediated transport of (R)-, (S)-, (R,S)-methadone, LAAM and their main metabolites. Pharmacology. 2007;80(4):304-11.

40. Goldberg RJ, Bengtson J, Chen ZY, Anderson KM, Locati E, Levy D. Duration of the QT interval and total and cardiovascular mortality in healthy persons (The Framingham Heart Study experience). Am J Cardiol. 1991;67(1):55-8.

41. Girardin FR, Gex-Fabry M, Berney P, Shah D, Gaspoz JM, Dayer P. Drug-induced long QT in adult psychiatric inpatients: the 5-year cross-sectional ECG Screening Outcome in Psychiatry study. Am J Psychiatry. 2013;170(12):1468-76.

42. Romero J, Baldinger SH, Goodman-Meza D, Engstrom K, Valencia CR, Golive A, et al. Drug-induced torsades de pointes in an underserved urban population. Methadone: is there therapeutic equipoise? J Interv Card Electrophysiol. 2016;45(1):37-45.

43. Krantz MJ, Martin J, Stimmel B, Mehta D, Haigney MC. QTc Interval Screening in Methadone Treatment. Ann Intern Med. 2009;150(6):387-95.

44. Meili D, Broers B, Beck T, Bruggmann P, Hämmig R. Recommandations médicales pour les traitements basés sur la substitution (TBS) de la dépendance aux opioïdes 2012. Societé Suisse de Médicine de l'addiction, SSAM; Bern, Suisse. http://www.ssam.ch/SSAM/sites/default/files/Recommandations%20TBS%205%2003%202013.pdf. Accessed 14 Aug 2015.

Table 1: Demographic characteristics of the study population.

Characteristic	Value
Sex (n=251) Men Women	 190 61
Age (years) (n=251) Mean ± SD	 36.4 ± 8
Body weight (kg) (n=250) Mean ± SD	 77 ± 17
Body Mass Index (kg/m^2) (n=249) Mean ± SD	 25 ± 5
α-1 glycoprotein level (g/l) (n=181) Mean ± SD	 0.97 ± 0.31
Methadone dose (mg/j) (n=251) Mean ± SD Median (range; interquartile range)	 123 ± 75 120 (3-400 ; 60-160)
***(R)*-Methadone plasma concentrations (ng/ml)** (n=507) Mean ± SD (range) **Peak concentrations 1-5 h** (ng/ml) Mean ± SD **Trough concentrations 18-30 h** (ng/ml) Mean ± SD	 267 ± 168 (11-1129) 324 ± 184 (32-1129) 222 ± 140 (11-874)
***(S)*-Methadone plasma concentrations (ng/ml)** (n=507) Mean ± SD (range) **Peak concentrations 1-5 h** (ng/ml) Mean ± SD **Trough concentrations 18-30 h** (ng/ml) Mean ± SD	 264 ± 172 (11-1123) 344 ± 185 (47-1123) 192 ± 128 (11-717)
QTc interval (ms) (n=317) Mean ± SD (range) At time=1-5 h (ms) At time=18-30 h (ms)	 422 ± 24 (348-466) 410 ± 22 413 ± 22
Heart rate (bpm) (n=317) Mean ± SD	 71 ± 13
Albumin (g/l)(n=184) Mean ± SD	 46 ± 5
AST (U/l) (n=182) Mean ± SD	 58± 95
ALT (U/l) (n=182) Mean ± SD	 69± 87
γ-GT (U/l) (n=182) Mean ± SD	 73 ± 135
Potassium levels (mmol/l) (n=182) Mean ± SD	 4.5 ± 0.41
Total calcium levels (mmol/l (n=182) Mean ± SD	 2.3 ± 0.17

Co-Medication affecting the PK	
CYP3A4 strong inhibitors	1
CYP3A4 weak inhibitor	31
CYP3A4 and CYP2B6 strong inducers	4
CYP3A4 weak inducers	10
CYP2B6 weak inhibitors	2
CYP2D6 strong inhibitors	32
CYP2D6 weak inhibitors	49
Co-Medication affecting the QT	
Known risk of torsage de pointes	19
Possible risk of torsde de pointes	67
Conditional risk of torsade de pointes	39

Abbreviations : AST, aspartate aminotransferase ; ALT, alanine amino transferase ; γ-GT, gamma glutamyl transferase ; CYP, cytochrome P450.

Comedications affecting the PK [26-27]

CYP3A4 strong inhibitor: nefadozone (n=1)

CYP3A4 weak inhibitors : efavirenz (n=2), fluoxetine (n=10), fluvoxamine (n=3), quetiapine (n=8), valproate (n=6), desogestrel and/or ethinylestradiol (n=2)

CYP3A4 and CYP2B6 strong inducers : carbamazepine (n=1), efavirenz (n=2), St John's wort (n=1)

CYP3A4 weak inducers : topiramate (n=10)

CYP2B6 weak inhibitors : efavirenz (n=2)

CYP2D6 strong inhibitors : bupropion (n=1), fluoxetine (n=10), paroxetine (n=10), levomepromazine (n=5), metoclopramide (n=2), thioridazine (n=4)

CYP2D6 weak inhibitors : citalopram (n=9), escitalopram (n=12), fluvoxamine (n=3), haloperidol (n=1), hydroxyzine (n=1), nefadozone (n=1), quetiapine (n=8), risperidone (n=5), sertraline (n=4), venlafaxine (n=5)

Comdedications affecting the QT [28]

Known risk: haloperidol (n=1), astemizole (n=1), thioridazine (n=4) , citalopram (n=13)

Possible risk: venlafaxine (n=5), olanzapine (n=15), mirtazapine (n=32), tizanidine (n=1), quetiapine (n=8), risperidone (n=4), aripiprazole (n=1), trimipramine (n=1).

Conditional risk: paroxetine (n=10), chloral hydrate (n=10), fluoxetine (n=7), hydroxyzine (n=1), loperamide (n=1), pantoprazole (n=2), sertraline (n=4), amisulpride (n=3), doxepine (n=1).

Table 2 : Summary of the tested genetic polymorphisms in the study population (decrease or loss of function (LOF/DOF) and gain of function (GOF).

Gene	Alleles (Reference SNP)	Ref (n)	Het LOF/DOF (n)	Hom LOF/DOF (n)
CYP2B6	**6* (**4* (rs2279343) and **9* (rs3745274))	146	84	14
	**5* (rs3211371)	184	57	3
	**11* (rs35303484)	240	4	0
	Intronic (rs2279344)	99	114	31
	intronic (rs8192719)	145	82	17
CYP2B6 activity score[#]	Combination of **5, *6, *11*	143	86	15
CYP2C9	**2* (rs1799853), **3* (rs1057910)	160	73	11
CYP2C19	**2 (*rs4244285*), *3* (rs4986893)	169	63	12
CYP2D6	**3* (rs35742686) ,**4* (rs3892097), **6* (rs5030655), **5* (gene deletion)	121	108	11
CYP3A4	**1B* (rs2740574)	223	20	1
	**22* (rs35599367)	221	23	0
CYP3A5	**3* (rs776746)[§]	2[§]	37[§]	205[§]
CYP3A7	**1C* (rs11568826 and rs11568825)	226	18	0
ABCB1	*61A>G* (rs9282564)°	201	36	7
ABCB1	*1199G>A* (rs2229109)°	218	25	1
ABCB1	*1236C>T* (rs1128503)	48	119	77
ABCB1	*2677G>T/A* (rs2032582)	54	109	81
ABCB1	*3435C>T* (rs1045642)	61	127	56
		Ref (n)	**Het GOF (n)**	**Hom GOF (n)**
POR	**28* (rs1057868)°	133	106	12
CYP2D6	**xN*		11	

Abbreviations. LOF: loss of function; DOF: diminished function; GOF: gain of function; Ref: Reference, Het: heterozygote, Hom: homozygote**.**

[#] Combination of *CYP2B6*6, *5* and **11* alleles: a score of 2 (Ref) was attributed to fully functional alleles (*CYP2B6 *1/*1, *1/*4, *1/*5, *4/*5, *5/*5),* a score of 1 (Het LOF/DOF**)** to *CYP2B6 *1/*11, *1/*6, *4/*6, *5/*6* and a score of 0 (Hom LOF/DOF) to *CYP2B6 *6/*6* and **6/*11*.

[§] Ref genotype corresponds to *CYP3A5 *1/*1*, Het LOF to *CYP3A5 *1/*3* and Hom LOF to *CYP3A5 *3/*3.*

° Effect might be substrate-dependant.

Table 3: Final population pharmacokinetic and pharmacodynamics parameters for *(R)*- and *(S)*-methadone with the parameters obtained from the 2000 bootstrapped samples.

	Final Population estimates				**Bootstrap** (n=2000 samples)			
Parameters	***(R)*-Methadone**		***(S)*-Methadone**		***(R)*-Methadone**		***(S)*-Methadone**	
Pharmacokinetics	**Estimate**	**RSE (%)** [a]	**Estimate**	**RSE (%)** [a]	**Mean**	**RSE (%)** [a]	**Mean**	**RSE (%)** [a]
k_a (h^{-1})	0.46	--	0.85		0.46	--	0.85	--
CL (L/h)	7.73	10%	7.31	12%	7.47	9%	7.27	12%
Q (L/h)	79.8	6%	69.1	34%	81.6	12%	72.4	26%
Vc (L)	105	18%	126	31%	102	25%	151	22%
Vp (L)	655	15%	229	13%	774	43%	209	40%
Tlag (h)	0.7	--	0.39	--	0.7	--	0.39	
θ_{AAG} (L)	--	--	0.23	12%	--	--	0.24	25%
$\theta_{CLCYP2B6}$ (L/h)	1.47	44%	3.10	15%	1.52	35%	3.06	15%
$\theta_{CL\ POR*28\ TT/CT}$ (L/h)	1.33	40%	--	--	1.32	40%	--	--
$\theta_{CL\ ABCB1\ 3435\ CC/CT}$(L/h)	--	--	-1.30	55%	--	--	-1.25	60%
$\theta_{CL\ CYP3A4*22\ TT/CT}$ (L/h)	-1.81	42%						
Pharmacodynamics								
Intercept (ms)	405	1%	407	1%	404	1%	406	1%
Slope(ms/1000ng.ml^{-1})	9.9	36%	19.2	56%	11.2	41%	19.0	34%
Interindivdiual variability (CV%) [b]								
ω_{CL}	41%	4% [c]	51%	6% [c]	41%	6%	51%	6%
ω_{Vc}	62%	19% [c]	71%	21% [c]	63%	18%	55%	22%
Correlation CL-Vc	81%	19% [c]	74%	13% [c]	--	--	--	--
ω_{Q}	37%	9% [c]	67%	31% [c]	38%	27%	76%	20%
ω_{ka}	--	--	--	--				
$\omega_{Intercept}$	4%	9% [c]	4%	7% [c]	4%	7%	4%	7%
Intraindividual variability (CV %) [d]								
$\sigma_{conc.}$	25%	6% [c]	28%	17% [c]	6%	6% [c]	27%	13%
σ_{QT}	3%	9% [c]	3%	7% [c]	3%	7% [c]	3%	16%

Final model for (*R*)-methadone: $TVCL = CL + \theta_{CLCYP2B6} \cdot \sqrt{n} + \theta_{CLPOR*28\,TT/CT} + \theta_{CLCYP3A4*22\,CT}$;
and for (*S*)-methadone $TVCL = CL + \theta_{CLCYP2B6} \cdot \sqrt{n} + \theta_{\text{CL ABCB1 3435 CC/CT}}$

Abbreviations: TV, typical population value; CL, clairance; Q intercompartmental CL; V_c and Vp, volume of distribution of the central et peripheral compartment, respectively; ka, absorption rate constant; Tlag, lag time in drug absorption; $\theta_{AAG,}$ relative contribution of α acid-glycoprotein (AAG) on the volume of distribution; $\theta_{CLCYP2B6,}$ relative contribution of cytochrome 2B6 coded as an activity score (see text) on CL; $\theta_{\text{CL POR*28 TT/CT,}}$ relative contribution of cytochrome *POR*28* heterozygotes and homozygotes on CL; $\theta_{\text{CL ABCB1 3435 CC/CT}}$, relative contribution of *ABCB1 3435 C>T* heterozygotes and homozygotes on CL; $\theta_{\text{CL CYP3A4*22 CT;}}$ relative contribution of *CYP3A4*22* heterozygotes on CL.

[a] Relative standard error of estimates (RSE), calculated as SE/estimate.
[b] Coefficient of variation, calculated as $\sqrt{e^{(\omega^2)}-1}$.
[c] Relative standard error of the estimates, calculated as SE/RSE with SE= $(SE^{(\omega^2)}/2\sqrt{\omega})$.
[d] Coefficient of variation, calculated as $\sqrt{e^{(\sigma^2)}-1}$.

Clinical Pharmacokinetics

Population Genetic-Based Pharmacokinetic Modeling of Methadone and Relationship with the QTc Interval in Opioid Dependent Patients

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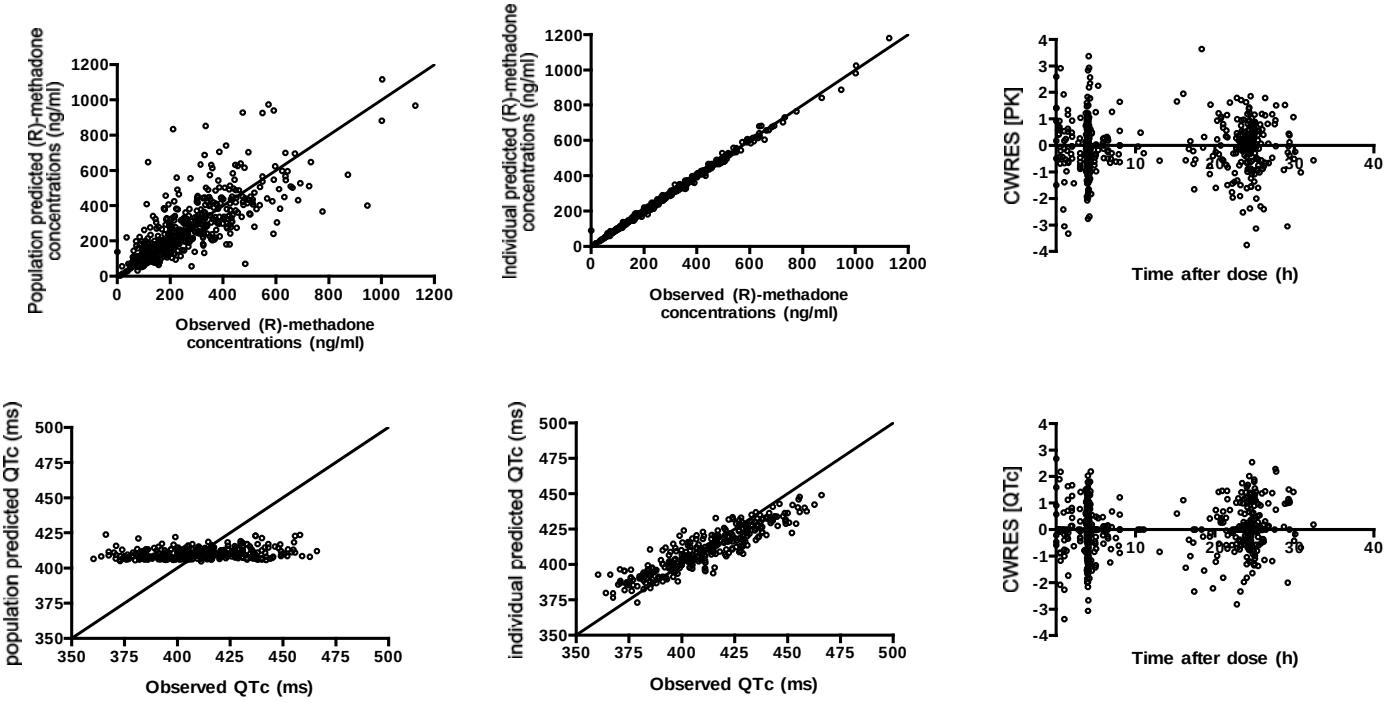
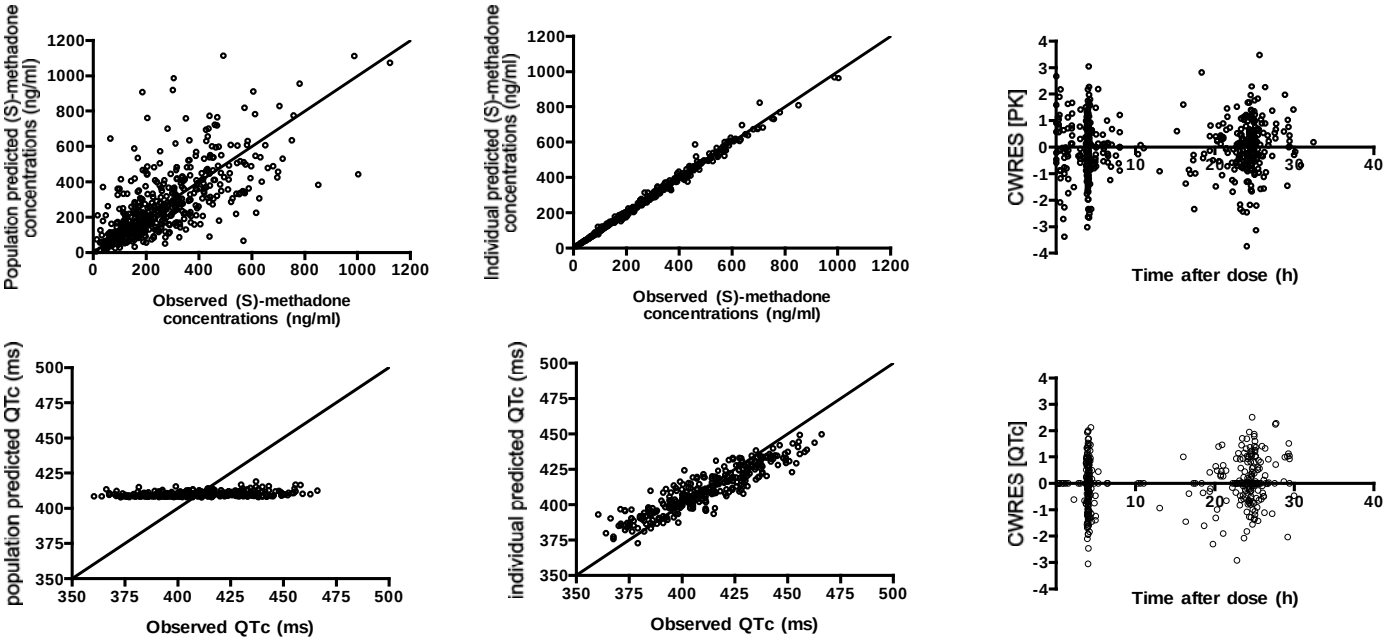
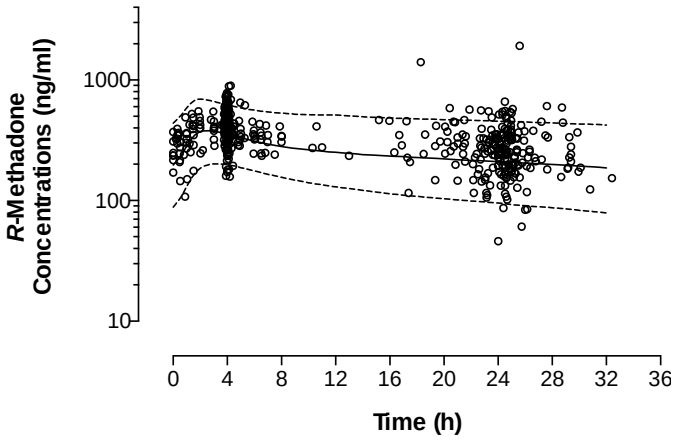
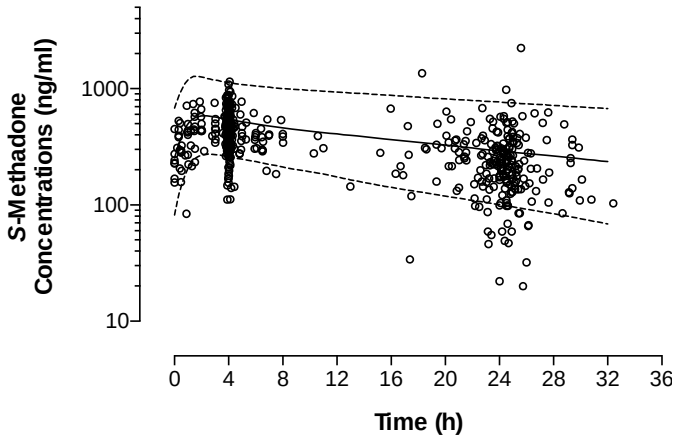
A

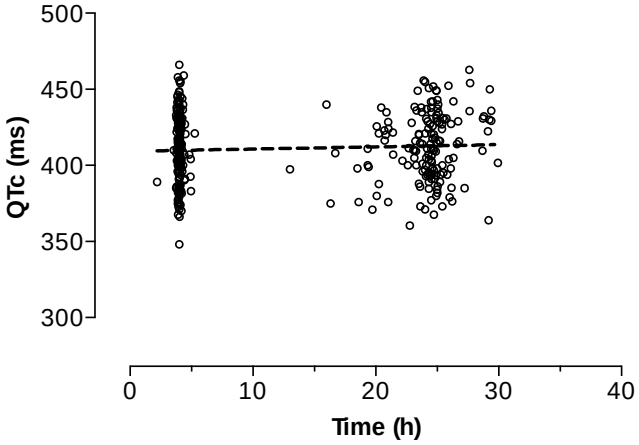


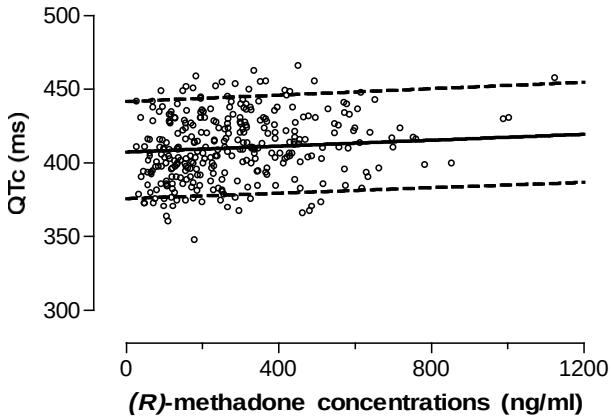
B

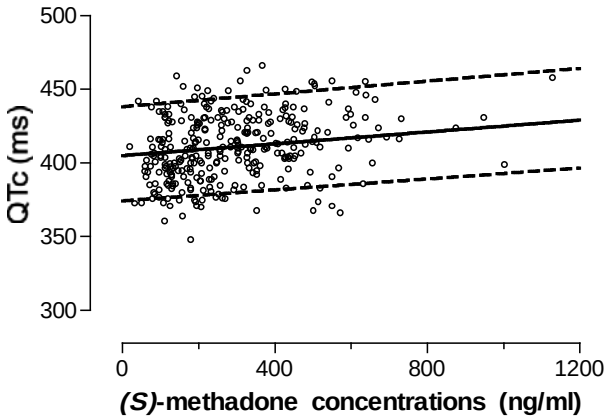



R-Methadone Concentrations (ng/ml)
1000
100
10
0
4
8
12
16
20
24
28
32
36
Time (h)

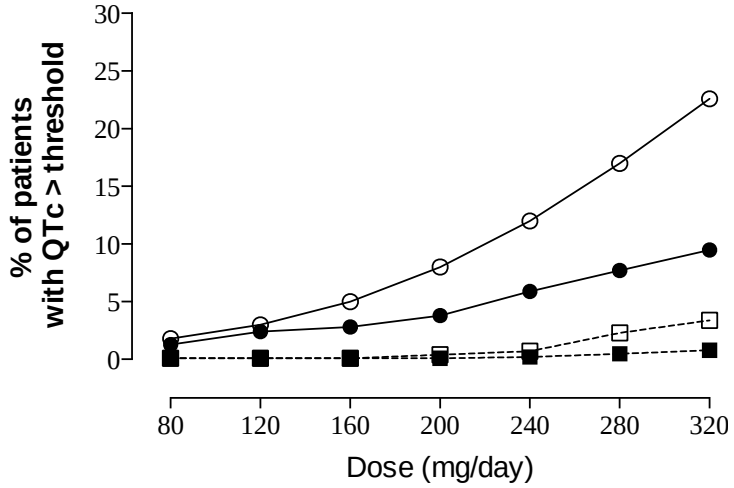

S-Methadone Concentrations (ng/ml)
1000
100
10
0
4
8
12
16
20
24
28
32
36
Time (h)


QTc (ms)
500
450
400
350
300
0
10
20
30
40
Time (h)

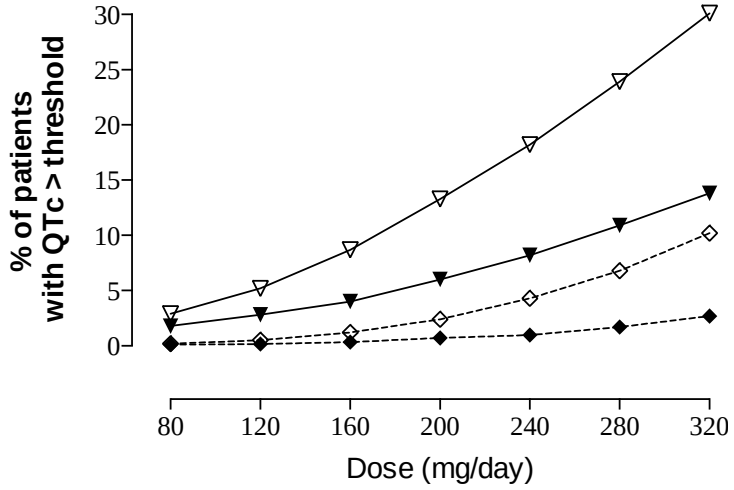

QTc (ms)
500
450
400
350
300
0
400
800
1200
(R)-methadone concentrations (ng/ml)


QTc (ms)
500
450
400
350
300
0
400
800
1200
(S)-methadone concentrations (ng/ml)

A. (*R*)-methadone



B. (*S*)-methadone



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