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ORIGINAL RESEARCH ARTICLE

Population Pharmacokinetic Quantification of CYP2D6 Activity in Codeine Metabolism in Ambulatory Surgical Patients for Model-Informed Precision Dosing

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Abstract

Background and Objective Codeine metabolism in humans is complex due to the involvement of multiple cytochrome P450 (CYP) enzymes, and has a strong genetic underpinning, which determines the levels of relevant CYP450 enzyme expression in vivo. Polymorphic CYP2D6 metabolises codeine to morphine via O-demethylation, while a strong correlation between CYP2D6 phenotype and opioidergic adverse effects of codeine is well documented. The aim of this study was to quantify the effect of *CYP2D6* genotype on the biotransformation of codeine.

Methods We conducted a prospective clinical trial with 1000 patients, during which ambulatory patients were administered 60 mg of codeine preoperatively and the association between CYP2D6 activity and morphine exposure across various *CYP2D6* genotypes was quantified using a population pharmacokinetic model. Plasma concentration data for codeine and its primary metabolites were obtained from 997 patients and *CYP2D6* genotype was screened for study subjects, and respective sums of activity scores assigned for each CYP2D6 allele were used as covariates in model development.

Results Our final model predicts the disposition of codeine and the formation of morphine, codeine-6-glucuronide and morphine-3-glucuronide adequately while accounting for variability in morphine exposure on the basis of *CYP2D6* genotype. In agreement with previous results, patients with decreased function alleles (*CYP2D6*10* and **41*) showed varying levels of decrease in CYP2D6 activity that were inconsistent with increasing activity scores. Model simulations demonstrate that morphine concentrations in ultrarapid CYP2D6 metabolisers reach systemic concentrations that can potentially cause respiratory depression (over 9.1 ng/mL), and have 218% higher exposure (19 versus 8.7 µg · h/L, $p < 0.001$) to morphine than normal metabolisers. Similarly, poor and intermediate metabolisers had significantly reduced morphine exposure (1.0 and 3.7 versus 8.7 µg · h/L, $p < 0.001$) as compared with normal metabolisers.

Conclusions Our final model leads the way in implementing model-informed precision dosing in codeine therapy and identifies the use of genetic testing as an integral component in the effort to implement rational pharmacotherapy with codeine.

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Key Points

The analgesic properties of codeine are dependent on CYP2D6-mediated biotransformation to morphine. *CYP2D6* genetic polymorphism results in marked changes in morphine exposure in humans. Current Clinical Pharmacogenetics Implementation Consortium guidelines have devised an activity scoring system that can be used for CYP2D6 genotype–phenotype translation.

CYP2D6 metaboliser phenotype categories include patients with markedly different CYP2D6 activities, thereby causing inaccuracies in phenotype-based predictions. In patient populations, model-informed drug dosing can be used to extrapolate morphine exposure after codeine administration.

Genetic screening and the quantitative representation of *CYP2D6* genotype might ensure the safety of drugs with variable dispositions on the basis of genetic profiles.

1 Introduction

It has been estimated that no less than 30% of day-case surgery patients suffer from severe or moderate pain at 24 h after surgery [1, 2]. Multi-modal analgesia with codeine, paracetamol and non-steroidal analgesic agents has proven effective in treating severe postoperative pain after ambulatory surgery. Codeine is a prodrug that is metabolised to morphine via cytochrome P450 2D6 (CYP2D6), which is followed by a UGT2B7 to biologically inactive morphine-3-glucuronide and active analgesic morphine-6-glucuronide representing approximately 90% and 10% of morphine first-order elimination, respectively. The *CYP2D6* gene is highly variable, and different genotypes of *CYP2D6* lead to wide interpatient variation in metabolic activity and consequent drug disposition [3–6].

The effect of *CYP2D6* genotype on the analgesic effect of codeine has been demonstrated in various experimental and clinical settings [7–11]. Individuals lacking *CYP2D6* activity, that is, poor metabolisers, suffer from poor analgesia from codeine, whereas ultrarapid metabolisers may experience exaggerated and potentially life-threatening adverse effects [7, 8]. The association of CYP2D6 phenotype with morphine-linked adverse effects is well known, while the in vivo activities of various *CYP2D6* allelic variants and genotypes have not been quantified during codeine therapy.

CYP2D6 genotypes are commonly translated into four metaboliser phenotypes: poor metabolisers (PM), intermediate metabolisers (IM), normal metabolisers (NM) and ultrarapid metabolisers (UM) [5, 6]. The CYP2D6 activity score assigns each allele an activity score value, and the sum of these forms the activity score for each genotype [12]. Activity score ranges are then used to define four CYP2D6 phenotype classes. Activity scores can also be used without phenotype classification to more precisely capture CYP2D6 activity [5, 13, 14]. Several studies have evaluated the assignment of *CYP2D6* alleles with different substrates in clinical settings [13–16]. However, most of these studies are retrospective and have evaluated pooled data from several small studies with a limited number of *CYP2D6* allele variants (e.g. *CYP2D6*10*).

We conducted a prospective clinical trial with 1000 patients, during which ambulatory patients were administered 60 mg of codeine preoperatively. Our aim was to quantify the effect of *CYP2D6* genotype on the biotransformation of codeine. To achieve this aim, we developed a population pharmacokinetic model using plasma codeine, morphine, codeine-6-glucuronide (C6G) and morphine-3-glucuronide (M3G) plasma concentrations and *CYP2D6* genotype data.

2 Method

2.1 Study Protocol

Data from a prospective clinical study with 1000 patients undergoing ambulatory surgery were analysed. The study was registered to EudraCT (no. 2015-005561-23) and approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District (3/2016) and the Finnish National Agency for Medicines (KL no. 4/2016). The data were collected at the day-surgery unit of Jorvi Hospital, University of Helsinki, and Helsinki University Hospital, Espoo, Finland from August 2016 to March 2018. When the patients were recruited to the study, medication used at home before surgery was recorded. Use of strong inhibitors of CYP2D6 was an exclusion criterion. All study patients received oral premedication with paracetamol (1000 mg) and codeine (60 mg) for preoperative prophylactic analgesia. After pre-medication, two blood samples were obtained through a cannula placed in the forearm vein. The first blood sample was drawn after 20–60 min and the second sample was drawn after 180–360 min after receiving the paracetamol-codeine combination tablets. The time of blood sampling was adapted to the progress of the treatment process, and the blood samples were commonly taken during

the induction of anaesthesia and at the time of discharge from hospital.

The adverse effects of pain medication, such as nausea and vomiting (yes or no), dizziness, sleepiness and constipation (score from 0 to 3) were asked repeatedly in the recovery room after surgery and after discharge from hospital twice daily until 2 days after the surgery. Use of antiemetics in the recovery room was also recorded. Detailed information about the study protocol and conduct has been provided in Online Resource 1.

2.2 Drug Analysis

Plasma concentrations of codeine, morphine and the glucuronide conjugates of codeine and morphine were determined with high-performance liquid chromatography-tandem mass spectrometry. Morphine, codeine, morphine 6-glucuronide, codeine-6-glucuronide and stable labelled morphine-D6 and codeine-D6 were purchased from Toronto Research Chemicals (Toronto, Canada). Prior to analysis, the plasma samples were pre-treated using a Strata X-C solid phase extraction in a 96-well format (Phenomenex, Torrance, CA, USA). Briefly, the samples (0.1 mL) were diluted with 0.15 mL of 4% phosphoric acid containing the internal standards and loaded into the preconditioned extraction wells. The wells were then washed with 0.15 mL of 2% formic acid, followed by 0.15 mL of methanol, and the analytes were eluted using 0.15 mL of 5% ammonium hydroxide in methanol. Finally, the sample extracts were dried using a centrifugal evaporator (GeneVac, Thermo Fisher Scientific, USA) and reconstituted in 0.1 mL of 5% methanol.

All measurements were carried out using a Sciex 6500 Qtrap LC-MS system interfaced with an electrospray ion source (Sciex, Toronto, Ontario, Canada). The analytes were separated on a Kinetex biphenyl column (2.6 µm particle size, 2.1 × 100 mm internal diameter; Phenomenex, Torrance, CA, USA) under gradient conditions and a 0.28 mL/min flow rate. The mobile phase consisted of a mixture of 0.1% acetic acid (solvent A) and acetonitrile-methanol (20:80 v/v) (solvent B), and the solvent gradient was set as follows: a linear ramp from 5 to 30% B over 3.5 min, a second linear ramp from 70 to 90% B over 1.5 min and 3 min equilibration back to the initial eluent composition. The mass spectrometer was operated in positive polarity mode, and the mass-to-charge (m/z) transitions used for quantification were 286–152 for morphine, 462–286 for morphine 6-glucuronide, 300–152 for codeine and 476–300 for codeine-6-glucuronide. The limit of quantification was 0.05 ng/mL, except for morphine-6-glucuronide (0.1 ng/mL). The day-to-day coefficients of variation (CV) values were below 11% at relevant concentrations for all analytes.

2.3 Genotyping

Genotyping was carried out using TaqMan^{R} genotyping and copy number assays on the QuantStudio™ 12K Flex Real-Time PCR System or targeted next-generation sequencing using the Ion GeneStudio™ S5 Prime system (Thermo Fisher Scientific, Waltham, MA). All samples were genotyped for the clinically relevant *CYP2D6* sequence variations, defining the **1-, *2-, *3-, *4-, *5-, *6-, *9-, *10-, *15-, *17-, *35-, *39-* and **41*-alleles, as well as for *CYP2D6* copy number variation (CNV). The *CYP2D6*-genotype distribution in the study population is shown in additional data provided in Online Resource 2. CYP2D6 phenotypes were inferred from the genotypes using the activity scores on the basis of recent consensus [5, 6, 12]. A detailed description of the genotyping is found in the Online Resource 1.

2.4 Pharmacokinetic Modelling

Non-linear mixed effects modelling was conducted with NONMEM® (version 7.4.3 or above) (ICON Development Solutions, Ellicott city, MD, USA) [17], assisted by the Perl-Speaks-NONMEM (PsN)-toolkit [18].

The pharmacokinetic modelling of concentration–time data was conducted in a stepwise manner. First, a pharmacokinetic model was developed and validated for codeine. Next, we included morphine data in the dataset and tested parent-metabolite disposition models. The C6G and M3G data were then added in the analysis data in a sequential manner to improve model performance in accounting for the complex enzymatic interplay defining codeine and morphine elimination (Fig. 1). Finally, the influence of CYP2D6 activity scores (AS) was added to the model, and several parameterisations were tested. The influence of the remaining observable patient characteristics (covariates) on codeine/morphine metabolism and disposition profiles was tested using the stepwise-covariate-modelling tool in the PsN toolkit [18]. Detailed description of the modelling process and the differential equations derived from the final model are given in the Online Resource 1.

Models were coded using differential equations and solved by the *ADVAN13* subroutine of the NONMEM® software. The model parameters were modelled in the log-domain using MU-referencing and computed using the first-order conditional estimation with interaction estimation method.

Exponential model was used to characterise between-subject variability in model parameters, and the covariance between the random effect parameters was also tested. The residual variability in the model predictions was specified using an additive error model. Both were assumed to be

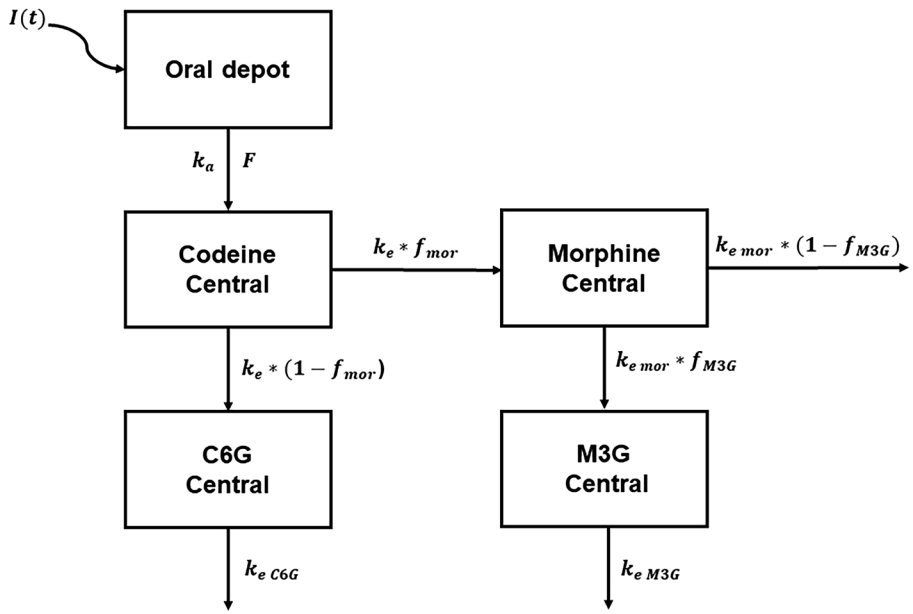

Fig. 1 Schematic of the final structural model for codeine and its metabolite morphine. The pharmacokinetics of codeine and morphine are described with one-compartmental mammillary models with the first-order absorption of codeine. The total clearance of codeine (CL_{cod}) is divided into CYP2D6- ($k_e \times f_{mor}$) and non-CYP2D6-mediated [$k_e \times (1 - f_{mor})$] clearance. CYP2D6-mediated clearance is used as an input in the C6G model, of which 60% is subsequently metabolised to M3G ($k_{e,mor} \times f_{M3G}$) and 40% is metabolised via other pathways [$k_{e,mor} \times (1 - f_{M3G})$]. *I(t)*, oral dose; *F*, oral bioavailability; f_{mor}, ratio metabolised to morphine; k_e, elimination rate constant for codeine; MPR, metabolite-to-parent molecular weight ratio; $k_{e,mor}$, elimination rate constant for morphine; f_{M3G}, fraction of morphine metabolised to M3G; $k_{e,C6G}$, elimination rate constant for C6G; $k_{e,M3G}$, elimination rate constant for M3G

normally distributed random variables with a mean of zero and a SD of one.

Improvement in model fit was measured on the basis of significant differences in objective function value (OFV) and Akaike information criteria (AIC), the numerical stability of the model parameters, standard goodness-of-fit plots and visual predictive checks [19].

2.5 Covariate Model

The pharmacokinetic parameters defining clearance and the volume of the central compartment for both codeine and morphine were allometrically scaled to the population's median weight. The covariate effects of CYP2D6 activity on the ratio of codeine metabolised to morphine were tested sequentially using CYP2D6 phenotypes predicted from genotypes or activity scores to explain between-subject variability in codeine metabolism. Both categorical and continuous modelling approaches were tested, as described in Online Resource 1.

The stepwise covariate modelling protocol included in the PsN toolkit was used to test the influence of the additional patient covariates (age, cigarette smoking, and American Society of Anesthesiologists classification) on the fixed effects. For continuous covariates, power and exponential models were tested, as was a linear model specification for categorical covariates. Both forward and backward searches were performed, with search probabilities of 0.05 and 0.01, respectively.

2.6 Model Simulations

Model uncertainty was evaluated with the sampling importance resampling protocol [20] to determine the robustness of the final pharmacokinetic models. The protocol was run with 1000 samples and 500 resamples (M/m = 2). Secondly, the final pharmacokinetic model was used to simulate data for 1000 virtual study subjects who had designated activity scores and were receiving a clinically plausible range of codeine doses (from 30 mg o.d. for 1 day up to 60 mg q.i.d. for 4 days), using a typical population median body weight of 80 kg. Additionally, a new patient collective of 10,000 new patients was simulated using the covariate set of study subjects at codeine dose levels of 30 mg or 60 mg (t.i.d. or q.i.d.) to evaluate the effect of CYP2D6 phenotype on codeine/morphine disposition kinetics. Each phenotype class was simulated against varying codeine dosages. The non-compartmental analysis protocol in the PsN toolkit and *ncappc* R package was used to compute non-compartmental pharmacokinetic analysis.

3 Results

Altogether 1000 patients scheduled for ambulatory surgery were recruited into this study. Patients' mean (SD) age and weight were 47.8 years (12.9) and 79.8 (13.8) kg, respectively (Table 1). Concentration data for one or more analytes were missing from three patients, leaving 997 patients in the final dataset (Online Resource 1, Supplementary Fig. 1).

3.1 Pharmacokinetic Models

A one-compartmental pharmacokinetic model with a depot compartment and first-order absorption led to an adequate model fit (OFV = 211), with biologically plausible parameter estimates as compared with a two-compartmental model (ΔOFV = +2). Therefore, a one-compartmental model was chosen as the final structural model for codeine disposition. The addition of between-subject variability on the model parameters (k_a, V_{cent} and F) resulted in a significant OFV drop, with low shrinkage (< 30%) on the between-subject variability parameters. Finally, weight scaling was added on the clearance and volume parameters (ΔOFV = −55).

Next, a one-compartmental model for morphine was added to the model. Initially, the total systemic codeine first-order elimination was entirely directed as input into the metabolite model (OFV = 1224, AIC =1246). Serious numerical difficulties in estimating a biologically plausible value for morphine volume were noticed in this step of the modelling study. The addition of a fraction parameter (f_{mor}), denoting the proportion of codeine first-order elimination that gets converted to morphine, in conjunction with first-order codeine clearance, significantly improved model

Table 1 Characteristics of 1000 ambulatory surgical patients receiving preoperative oral 60 mg dose of codeine

	All patients	Predicted metabolizer status[b]			
		PM	IM	NM	UM
n	997	37	268	629	65
Age (years)	47.8 (12.9)	46.9 (12.2)	47.4 (13.1)	47.8 (12.8)	50.2 (13.8)
Weight (kg)	79.8 (13.8)	81.3 (12.8)	78.8 (14.1)	80.1 (13.6)	80.1 (14.4)
BMI (kg/m^2)	25.9 (3.60)	26.4 (3.04)	25.6 (3.93)	25.9 (3.48)	25.7 (3.58)
ASA-class (*n*)					
1	513 (51)	17	150	316	30
2	424 (43)	18	102	275	29
3	60 (6)	2	16	38	4
CYP2D6 activity score (*n*)[a]					
0	37 (3.7)	37	–	–	–
0.25	5 (0.5)	–	5	–	–
0.5	21 (2.1)	–	21	–	–
0.75	2 (0.2)	–	2	–	–
1	240 (24)	–	240	–	–
1.25	23 (2.3)	–	–	23	–
1.5	67 (6.7)	–	–	67	–
2	537 (54)	–	–	537	–
2.25	2 (0.2)	–	–	2	–
3	61 (6.1)	–	–	–	61
4	2 (0.2)	–	–	–	2

Values are mean (SD) for continuous parameters and number (proportion) for categorical characteristics

BMI, body-mass index; ASA, American Society of Anesthesiologists physical status classification system

[a]Predicted CYP2D6 activity. An AS value of 1 was assigned for each normal function allele (**1* and **2*), 0.5 for each decreased function allele (**9*, **17*, **29* and **41*), 0.25 for each **10* allele, and 0 for each no function allele (**3*, **4*, **5*, **6* and **40*). Duplicated normal function alleles in combination with CYP2D6*10 (**1x2/*10* and **2x2/*10*) were assigned as AS 2.25. Duplicated normal function alleles in combination with either a decreased function allele other than *CYP2D6*10 or another normal function allele were assigned as 3 (**1* or **2* with**1xN*, **2xN* or **35*) or 4 (**1xN* with **35xN* or **2xN*)

[b]Predicted CYP2D6 metabolizer status was inferred from the genotypes using the activity score (AS). AS of 0 indicates a poor metabolizer (PM), AS 0 < x < 1.25 indicates an intermediate metabolizer (IM), AS 1.25 ≤ x ≤ 2.25 indicates a normal metabolizer (NM), and AS of > 2.25 indicates an ultra-rapid metabolizer (UM)

performance (ΔOFV = −113, AIC = 733) and resulted in a biologically plausible model estimate (f_{mor} = 8%).

Finally, one-compartmental models were fitted for C6G and M3G, and a composite model was run with four analytes. The fraction of C6G from codeine elimination was numerically assigned as $1 - f_{mor}$, while the metabolic fraction of M3G from morphine elimination was fixed at 60%, according to a recent report [21]. This resulted in a precise model fit (ΔOFV = −1809, AIC = −1064) and biologically plausible parameter estimates.

3.2 Covariate modelling

The effect of CYP2D6 phenotype on codeine metabolism was modelled with activity scores, which were treated as a continuous covariate:

$$f_{\text{mor}} = \theta_{\text{A}} \times \exp\left(\theta_{\text{B}} \times \left(\text{AS}_{\text{ref}} - \text{AS}_i\right)\right),$$

where f_{mor} is the fraction of codeine metabolised to morphine. The individual value of an activity score (AS_i) is scaled to the reference value (AS_{ref}) of 2, and θ_A is the reference value of f_{mor}, while θ_B is the scaling factor for translating the effect of activity scores on f_{mor}.

CYP2D6-based genetic effect was modelled either through categorical effect of metaboliser status (i.e. PM, IM, NM or UM), with a separately estimated model parameter corresponding to value f_{mor} for each of these categories (ΔOFV = −711), or by treating AS as a continuous covariate and implementing a linear (ΔOFV = −2725), exponential (ΔOFV = −2970) or power (ΔOFV = −3556) model. Further details about these implementations have been provided in the ESM. Although power model was preferable in terms of OFV drop, repeated attempts to counteract a marked lack of precision in estimated model parameters (denoted by very high %RSEs) and numerical difficulties in model estimation (lack of convergence and retrieval of variance-covariance matrix) led to the power model being discarded possibly due to the addition of a separate model parameter for AS = 0, and exponential model was retained as the final model.

Prior to the addition of a CYP2D6 effect, the morphine/codeine ratio was estimated at 8%, with 52% unexplained variability. After the implementation of a covariate model, the unexplained variability was reduced to 45% and 33% after using the phenotype classes or activity scores as the covariate, respectively.

In addition to *CYP2D6* genotype, patient weight, age, height, American Society of Anesthesiologists physical activity score, smoking status and number of smoked cigarettes (if a smoker) were tested. There was no further improvement in the predictive performance of the final model. The final pharmacokinetic model (Fig. 1) provided an adequate fit to the study data (Fig. 2; Online Resource 1, Supplementary Figs. S2–S5), with biologically plausible parameter estimates for the pharmacokinetic and covariate model parameters and possessed numerical and predictive stability (Table 2).

3.3 Quantification of the Effect of Different Genotypes

The *CYP2D6* genotype data were available for all 997 patients included in the population pharmacokinetic analysis. A total of 64 *CYP2D6* genotypes were observed (see Online Resource 2).

The apparent total clearance of codeine is divided into CYP2D6- ($k_e \times f_{mor}$) and non-CYP2D6-mediated [$k \times (1 - f_{mor})$] metabolisms. CYP2D6-mediated metabolism reflects the formation of morphine, and Fig. 3A shows the apparent CYP2D6 activity for patients on the basis of their *CYP2D6* genotypes. The median (interquartile range, %) apparent CYP2D6 activity was 0.55 (0.34–0.75) for CYP2D6 PMs, 6.82 (5.39–8.67) for IMs, 13.8 (10.9–16.7) for NMs and 19.9 (16.8–23.1) for UMs of the total codeine clearance. Patients who were heterozygous for the *CYP2D6*10* allele seemed to have higher apparent CYP2D6 activity than patients heterozygous for the *CYP2D6*41* allele.

To evaluate the translation of genotype into metaboliser phenotype, *CYP2D6* genotypes were grouped according to activity scores (Fig. 3B). The activity score groups differentiated *CYP2D6* activities well according to their predicted activity, but the *CYP2D6*10* allele seemed to confer higher activity than expected from the activity scores.

CYP2D6 phenotype was statistically significantly associated to only one adverse effect of codeine, constipation. Slow metabolisers had more constipation, but there were no other statistically significant differences between the CYP2D6 phenotypes in the adverse effects.

3.4 Model Simulations

The sampling-importance resampling procedure [20] resulted in narrow confidence intervals for all model parameters, demonstrating robustness and precision (Table 2). Simulations for a typical patient with a clinically plausible postoperative codeine dosing regimen show that repeated dosing results in high morphine exposure in individuals exhibiting high CYP2D6 activity scores, that is, groups 3 and 4 (NM and UM, respectively), with systemic concentrations either reaching the EC50 for respiratory depression or exceeding it.

The results of the non-compartmental analysis are presented in Table 3. Simulated exposures for codeine and C3G were similar between different activity scores and metaboliser phenotypes, but AUC/F for both morphine and M6G differed significantly between the groups. There was

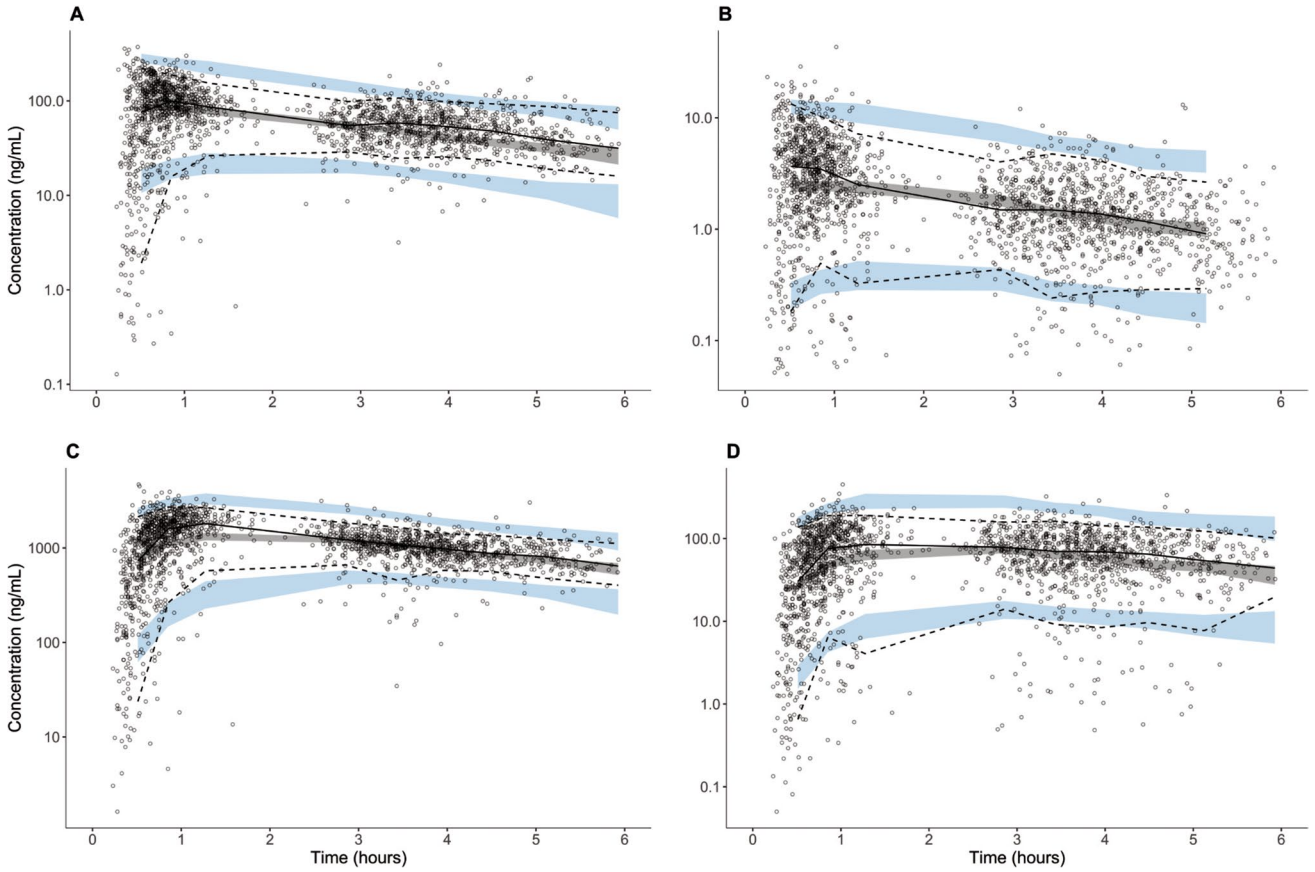

Fig. 2 Visual predictive checks. Visual predictive checks based on 1000 simulations showing **A** codeine, **B** codeine-6-glucuronide, **C** morphine and **D** morphine-3-glucuronide after 60 mg per oral codeine dose. Black circles represent individual observations. The black solid and dashed lines represent the observed median and the 5th and 95th percentiles of the observed plasma concentrations, respectively. The grey shaded area denotes the simulation-based 95% confidence interval for the median, and the 95% confidence intervals for the corresponding percentiles of the predictions are shown as the light blue shaded areas. Time refers to time after the codeine administration

a 20-fold difference in AUC/F between activity scores of 0 and 3 and over a two-fold difference between activity scores of 2 and 3. In the NM and IM groups, patients carrying the *CYP2D6*41* allele had consistently higher AUC/F as compared with patients carrying the *CYP2D6*10* allele.

Concentration-time profiles for 1000 new typical individuals were simulated to demonstrate the role of increasing activity scores in dynamically predicting morphine exposure (clinically plausible doses of 60 mg o.d.–q.i.d., Fig. 4). The simulated morphine concentrations were noticeably low (0–1 ng/mL) for CYP2D6 AS 0 and gradually approach the EC50 for respiratory depression at activity score 3 and cross this level in t.i.d. and q.i.d. dose-administration schemes. For activity score 4, morphine exposure continuously crosses the level of EC50 in all simulated dosing schemes. Additionally, a stochastic simulation with a new patient collective of 10,000 individuals and the study subjects' covariate set supported these results (Online Resource 1, Supplementary Fig. S6).

4 Discussion

Recently, several studies have evaluated the functional assignment of *CYP2D6* alleles with different substrates in clinical settings [13–16]. These analyses have provided support for the functional assignment of *CYP2D6* alleles, but these retrospective analyses have pooled data from separate studies or therapeutic drug-monitoring data. We conducted a prospective study in ambulatory surgical patients receiving a fixed dose of codeine preoperatively. A large dataset composed of 997 patients' PK data, with a diverse selection of relevant *CYP2D6* alleles, was analysed, and our primary aim was to quantify the in vivo effect of *CYP2D6* genotype on the metabolism of codeine.

Our results show a 58–83% reduction in apparent CYP2D6 activity via decreased function activity scores as compared with the normal metaboliser category activity scores (i.e. NM in panel c versus IM in panel b in Fig. 3B).

Table 2 Parameter estimates from the final pharmacokinetic model for codeine with median and 95% confidence intervals from the sampling importance resampling procedure with 1000 final samples and 2000 resamples

Parameter	Description[a]	Parameter estimate		SIR results	
		Median	95% CI[a]	Median	95% CI
$k_{a,cod}$	Codeine absorption rate constant (h^{-1})	8.74	(6.49–10.98)	8.81	(6.53–11.18)
CL_{cod}	Codeine total systemic clearance (L/h)	110	(59.6–160.4)	108.9	(80.9–137.6)
$V_{c,cod}$	Codeine central volume (L)	427.5	(231.2–623.8)	414.1	(320.9–577.8)
F_{cod}	Codeine systemic bioavailability	0.84	(0.4556–1.00)	0.83	(0.63–0.97)
CL_{mor}	Morphine total systemic clearance (L/h)	357.5	(178.2–536.9)	353.7	(256.9–451.6)
$V_{c,mor}$	Morphine central volume (L)	22.8	(9.447–36.2)	22.5	(13.3–31.8)
f_{mor}	Fraction of codeine metabolized to morphine[b]	0.16	(0.108–0.209)	0.16	(0.11–0.22)
GEN_{eff}	Activity score scaling parameter	1.00	(0.96–1.05)	1.00	(0.96–1.05)
CL_{C6G}	C6G total systemic clearance (L/h)	7.96	(4.28–11.66)	7.89	(5.91–9.86)
$V_{c,C6G}$	C6G central volume (L)	5.36	(2.88–7 .86)	5.36	(3.82–6.92)
CL_{M3G}	M3G total systemic clearance (L/h)	9.45	(4.67–14.21)	9.41	(6.90–11.82)
$V_{c,M3G}$	M3G central volume (L)	8.47	(4.17–12.78)	8.43	(5.91–10.86)
$\eta_{k_a,cod}$	IIV on codeine absorption rate constant	3.75	(2.96–4.55)	3.78	(3.03–4.58)
$\eta_{V_c,cod}$	IIV on codeine central volume	0.181	(0.158–0.204)	0.181	(0.159–0.205)
$\eta_{F_{cod}}$	IIV on codeine bioavailability	0.024	(0.019–0.029)	0.024	(0.019–0.029)
$\eta_{CL_{mor}}$	IIV on morphine clearance	0.062	(0.046–0.077)	0.061	(0.047–0.075)
$\eta_{R_{mor}}$	IIV on codeine to morphine metabolic ratio	0.334	(0.306–0.361)	0.334	(0.306–0.363)
ε_{cod}	RV for codeine observations	0.259	(0.242–0.275)	0.259	(0.241–0.275)
ε_{mor}	RV for morphine observations	0.149	(0.136–0.162)	0.149	(0.137–0.161)
ε_{C6G}	RV for C6G observations	0.103	(0.094–0.112)	0.103	(0.094–0.113)
ε_{M3G}	RV for M3G observations	0.096	(0.087–0.105)	0.096	(0.086–0.104)

The subjects were given a single 60 mg codeine dose preoperatively during ambulatory surgery

η, level 1 random effects parameters; ε, level 2 random effects parameters; SIR, sampling importance resampling; CI, confidence interval; IIV, inter-individual variability; RV, residual variability

[a]Confidence interval based on standard errors obtained by NONMEM (assuming-normal distribution of respective parameter, computed as $\theta \pm z_1 - \frac{\alpha}{2} \times SE$, where SE is the standard error provided by NONMEM and $z_1 - \alpha/2$ is the 0.95th quantile of the standard normal distribution)

[b]Scaled to activity score 2

Furthermore, patients with increased function that is, activity scores of 3 and 4 versus activity scores of 1.5 and 2, respectively, had approximately 2.1- and 1.69-fold higher apparent CYP2D6 activity as compared with patients with normal function, respectively. The guidelines for translating the genotype into phenotype used to individualise drug dosing are ambiguous. A recent study with a large amount of retrospective therapeutic drug-monitoring data demonstrated that, compared with normal *CYP2D6* alleles, the activity scores of the *CYP2D6*41* and *CYP2D6*9-10* alleles were estimated to be one-sixth and one-third as large, respectively [16]. The results of this highly powered study provide a solid basis for the translation of the *CYP2D6* genotype into a drug metabolic phenotype. In accordance with these results, our study demonstrates that the *CYP2D6*10* decreased function allele shows higher apparent CYP2D6 activity than the *CYP2D6*41* allele. Thus, our results further confirm the previous findings [16, 22, 23] comparing these alleles and challenge the consensus of assigning a lower activity score to *CYP2D6*10* than other decreased-function *CYP2D6* alleles.

A one-compartment empirical pharmacokinetic model for both codeine and its main metabolites could capture the data well. Linear first-order elimination could capture the codeine elimination well, and the model-estimated apparent clearance was in accordance with previous reports [24]. Our results show that codeine is quickly and nearly completely absorbed from the gastrointestinal tract, which is in good agreement with the previous literature [25] The amount of codeine eliminated to morphine was estimated using a ratio parameter, and we assumed that the remainder was glucuronidated to C6G. In conjunction, M3G was assumed to account for 60% of morphine metabolism on the basis of the previous literature [24], and the model fit demonstrated an adequate fit. The addition of M3G data into the model improved the performance of the morphine model in

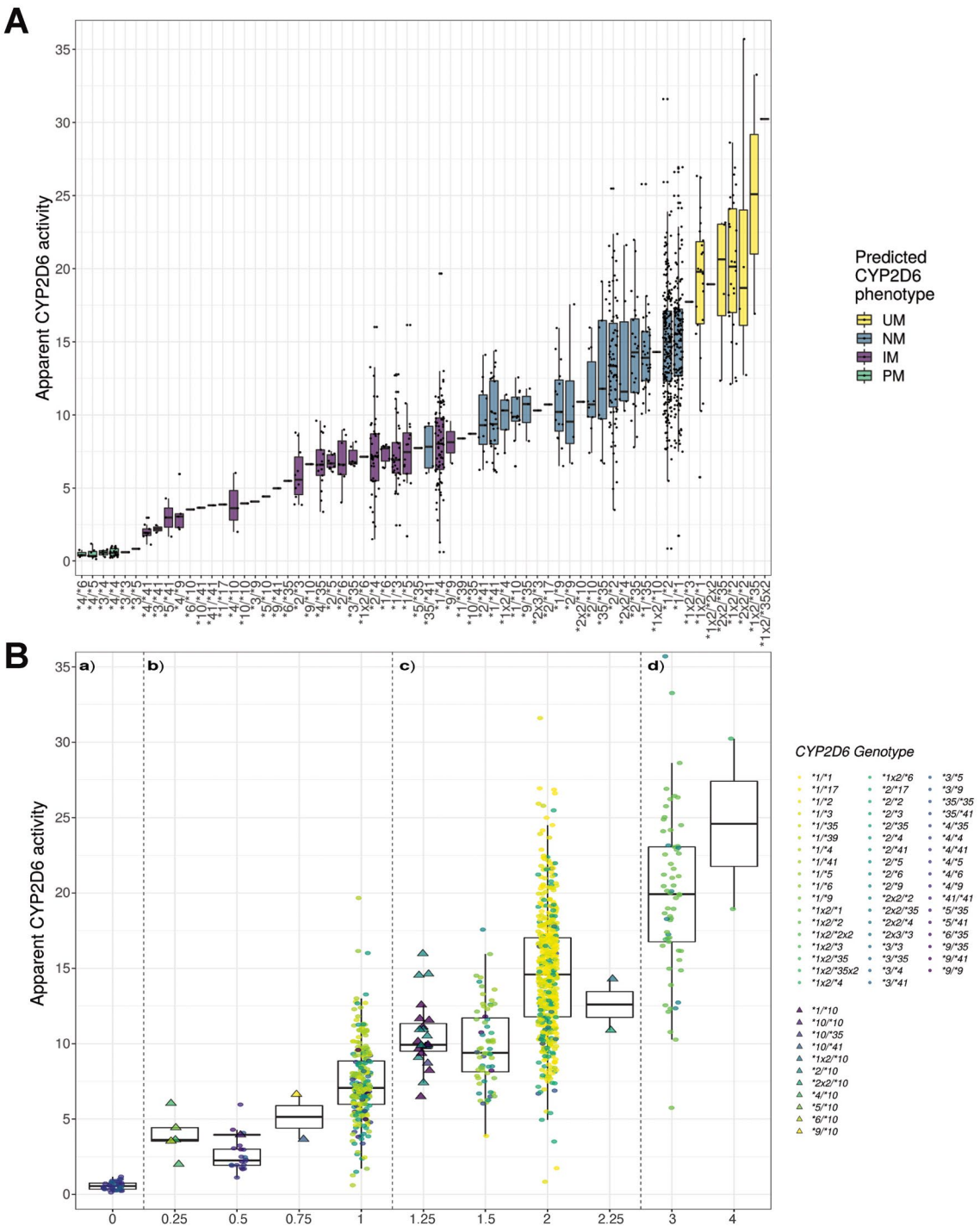

Fig. 3 The fraction of codeine metabolised to morphine, represented as apparent CYP2D6 activity ($f_{mor} \times CL_{CYP2D6}$), is shown as box- and scatterplots against **A** different *CYP2D6* genotypes and **B** activity scores, colour coded with predicted CYP2D6 metaboliser phenotypes. Patients carrying the *CYP2D6*10* allele are depicted with diamonds. IM, intermediate metaboliser; NM, normal metaboliser; PM, poor metaboliser; UM, ultrafast metaboliser. In B, predicted CYP2D6 phenotype-categories are separated with dashed lines

providing a plausible estimate for the apparent volume of distribution, which previously imparted numerical inconsistency to the parent/metabolite model.

Prior to the addition of a *CYP2D6*-effect, the morphine/codeine ratio was estimated at 8%, with 52% unexplained variability. After the implementation of a covariate model using CYP2D6 phenotype classes, the unexplained variability could be reduced to 45%, and after assigning CYP2D6 AS as the covariate, the unexplained variability was reduced further to 33%. This shows that there remains considerable

Table 3 CYP2D6 genotypes and predicted activity scores (AS) and CYP2D6 phenotypical classifications of 1000 ambulatory surgical patients studied

CYP2D6 genotype	*n* (%)	AS[a]	CYP2D6[b] phenotype	AUC/F (Codeine) (mg · h/L)	AUC/F (Morphine)(mg · h/L)	AUC/F (Codeine-6-Glucuronide) (mg · h/L)	AUC/F (Morphine-3-Glucuronide) (mg · h/L)
	63 (6.5%)		UM (> 2.25)	206 (121)	19 (14)	2482 (1333)	529 (351)
**1xN/*35xN, *1xN/*2xN*	2	4		193 (102)	31 (19)	1572 (832)	832 (512)
**1/*1xN, *1/*2xN, *1xN/*35, *2/*2xN, *2xN/*35*	61	3		207 (122)	19 (14)	2512 (1333)	519 (337)
	628 (63%)		NM (1.25 < x ≤ 2.25)	210.4 (121)	8.7 (7.3)	3187 (1570)	239 (180)
**1xN/*10, *2xN/*10*	2	2.25		206 (115)	11 (8.6)	3193 (1602)	330 (241)
**1/*1, *1/*2, *1/*35 , *1/*39, *1xN/*3, *1xN/*4, *1xN/*6, *2/*1, *2/*2, *2/*35, *2/*4, *2xN/*3, *2xN/*4, *35/*35*	537	2		211 (122)	9.2 (7.5)	3159 (1555)	243 (184)
**1/*17, *1/*41, *1/*9, *2/*17, *2/*41, *2/*9, *9/*35,, *35/*41*	67	1.5		198 (112)	5.7 (4.7)	3256 (1569)	163 (124)
**1/*10, *10/*2, *10/*35*	23	1.25		218 (132)	5.0 (4.4)	3643 (1826)	141 (112)
	268 (27%)		IM (0 < x < 1.25)	215 (125)	3.7 (3.4)	3568 (1747)	104 (84)
**1/*3, *1/*4, *1/*5, *1/*6, *2/*15, *2/*3, *2/*4, *2/*5, *2/*6, *3/*35, *4/*35, *41/*41, *5/*35, *6/*35, *9/*41, *9/*9*	240	1		214 (125)	3.9 (3.5)	3544 (1744)	108 (86)
**10/*41, *9/*10*	2	0.75		257 (125)	3.7 (2.9)	4412 (1833)	104 (78)
**10/*10, *3/*41, *3/*9, *4/*41, *4/*9, *5/*41*	21	0.5		219 (126)	2.5 (2.3)	3607 (1710)	66 (53)
**10/*4, *10/*5, *6/*10*	5	0.25		242 (133)	2.2 (1.8)	4429 (1820)	62 (48)
	37 (3.7%)		PM (= 0)	209 (120)	0.98 (1.3)	3602 (1738)	40 (33)
**3/*3, *3/*4, *3/*5, *4/*4, *4/*5, *4/*6*	37	0		209 (120)	0.98 (1.3)	3205 (1738)	40 (33)
Significance				0.198	< 0.001		< 0.001

Mean (SD) exposures for codeine and morphine are estimated on 1000 simulations on the basis of final pharmacokinetic model using *ncappc*-package

AS, activity score; AUC, area under the plasma concentration versus time curve; UM, ultra-fast metabolizer; NM, normal metabolizer; IM, intermediate metabolizer; PM, poor metabolizer

[a]Predicted CYP2D6 activity. CYP2D6 metabolizer status was inferred from the genotypes using the activity score (AS). An AS value of 1 was assigned for each normal function allele (*1 and *2), 0.5 for each decreased function allele (*9, *17, *29 and *41), 0.25 for each *10 allele, and 0 for each no function allele (*3, *4, *5, *6 and *40). Duplicated normal function alleles in combination with CYP2D6*10 (*1x2/*10 and *2x2/*10) were assigned as AS 2.25. Duplicated normal function alleles in combination with either a decreased function allele other than CYP2D6*10 or another normal function allele were assigned as 3 (*1 or *2 with*1xN *2xN or *35) or 4 (*1xN with *35xN or *2xN)

[b]AS of 0 indicates a poor metaboliser (PM), AS 0 < x < 1.25 indicates an intermediate metaboliser (IM), AS 1.25 ≤ x ≤ 2.25 indicates a normal metaboliser (NM), and AS of > 2.25 indicates an ultra-rapid metaboliser (UM)

[c]Significance was tested with exact (Permutation) version of the Jonckheere–Terptra test. It presumes a trend of decreasing AUC with increasing CYP2D6 genotype-based AS

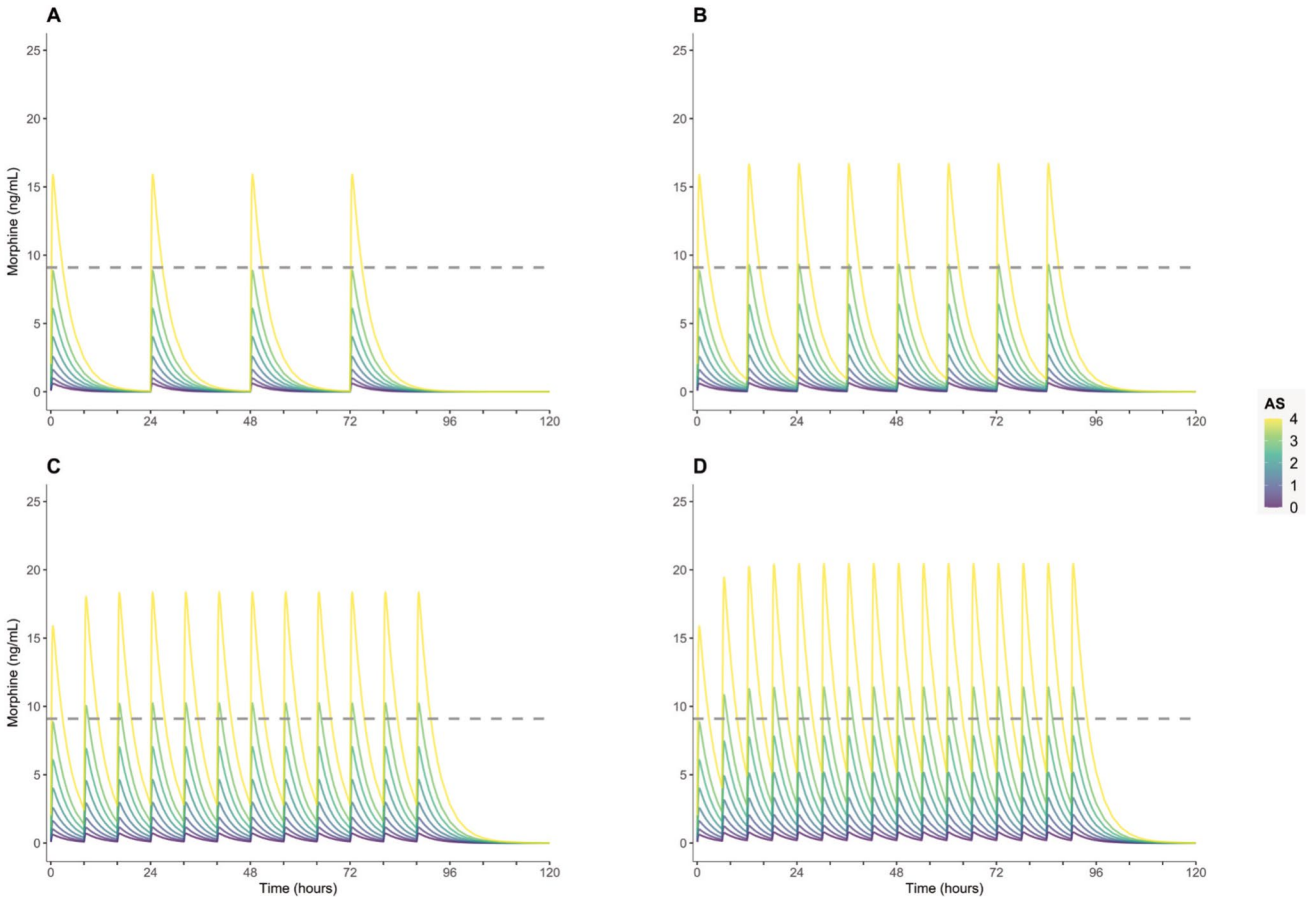

Fig. 4 The effect of *CYP2D6* genotype-based activity scores (AS) in clinically plausible dosing scenarios. Simulated plasma concentration profiles for a typical patient (70 kg) using the final model and oral codeine dose of 60 mg **a** once daily, **b** twice daily, **c** three times per day, and **d** four times per day. Colours show the effect of CYP2D6 activity scores (AS) on the concentrations. The dashed line indicates the previously reported 9.1 ng/mL EC50 value for morphine resulting in respiratory depression in 50% of patients

unexplained variability in the metabolic ratio parameter. A recent study showed that, while CYP2D6 AS explained 23% of the interindividual variability in CYP2D6 activity, there were considerable inconsistencies between different activity scores [26]. CYP2D6 protein level seems to be the major determinant of CYP2D6 activity according to this study [26]. Previous studies have demonstrated the importance of drug interactions [8], and underlying diseases and other physiological factors, together with the effect of the microbiome and inflammation, may further contribute to the expression and activity of CYP2D6 [27–29].

Morphine represents only a fraction of the total codeine metabolism in vivo, and previous results have shown that it accounts, on average, for only 5–10% of total codeine elimination clearance [30]. The *CYP2D6* genotype causes significant variability in this regard, and our results show that the median ratio metabolised to morphine varied from 0.5 to more than 30% in AS levels 0 and 4, respectively, demonstrating a progressive change dependent on the *CYP2D6* genotype. This translates into an almost 20-fold range between the AS groups during an average morphine exposure, as demonstrated by the simulated AUCs of morphine after 60 mg of codeine, averaging 1 µg·h/L in AS 0 and 19 µg·h/L in AS 4. These results are in accordance with previous clinical findings [5–8].

Coetzee [30] has devised a theoretical therapeutic window for morphine between 9 and 14 ng/mL in terms of its analgesic activity, in conjunction with a previously reported 9.1 ng/mL EC50 value for morphine in terms of producing analgesia and respiratory depression in 50% of patients [31]. The simulations derived from our final model show that the repeated administration of codeine can result in systemic concentrations either reaching EC50 for respiratory depression or exceeding it for AS 3 and 4, respectively. Additionally, our results show considerable variability in systemic codeine concentrations between individuals with the same

CYP2D6 genotype, indicating that some patients with AS < 3 may also exhibit dangerously high exposure to morphine after codeine administration.

It is worth noting that although there are significant activity differences between the allelic forms for *CYP2D6*, the median population level exposure to morphine (as denoted by morphine AUC/F in Table 3) follows the trend of increasing AS corresponding to higher morphine exposure in vivo. This supports the derivation of an AS-based system based on allelic genetic makeup, which as our modelling results suggest, is a more favourable way of individualising therapy with *CYP2D6* substrates as compared with conventional metabolic categories (PM, IM, NM and UM), but as our results also indicate, may require a careful re-evaluation of the assignment of activity scores to specific allelic components, and specifically the *CYP2D6*10* allele as per our investigation.

Although the activity scoring system for *CYP2D6* activity significantly improves the prediction of downstream metabolite (in this case morphine) exposure in comparison with metabolic categories of PM, IM, NM and UM by increasing complexity in *CYP2D6* activity-based classification, there might be subtle differences in the real-world activity of different functional alleles that may not be reflected in the current system of classification. It is clear from our analysis that while **10* allele imparts a higher functional *CYP2D6* activity as compared with the **41* allele (Fig. 3A), this effect gets masked with the pooling together of functional alleles under a specific cumulative activity score. For example, it is difficult to directly compare morphine exposure between the group of AS = 1.25 (constituted by allelic combinations of **1/*10*, **2/*10* and **10/*35*) against AS = 1.5 since the latter is composed of allelic combinations other than merely **1/*41*, **2/*41* and **35/*41* (Table 3). The other allelic combinations may lead to higher morphine exposure, and hence the subtle differences between **10* and **41* alleles in morphine exposure are masked when comparing only the median exposure per AS category. Therefore, it is important to keep in view relative differences between functional alleles when designing dosing schemes, especially for potent substances such as morphine for which the therapeutic window is quite narrow.

In this ambulatory surgery study, CYP2D6 phenotype was associated with only one adverse effect of codeine, constipation. The lack of other statistically significant differences in adverse effects of codeine between the CYP2D6 phenotype could be explained by short-term use of codeine; after the surgery it was used only if needed. Most of the patients did not have strong or long-lasting pain.

Despite a prospective study design and 1000 patients being recruited, only two blood samples were drawn from each patient for drug analysis. However, our analysis included all major metabolites together with the parent drug, and the model estimates show adequate precision and clinical plausibility. Furthermore, we determined a selection of 11 relevant *CYP2D6* alleles, together with copy number variation, to include 66 genotypes and thus quantify their effect. Although several other covariates were collected, we could not use them in our modelling, due to over-parameterisation issues. Thus, the effect of other covariates on codeine pharmacokinetics remains to be studied. We made several assumptions during the model development for the sake of simplicity, such as the fraction metabolised to M6G, but these were based on the previous literature. Although our findings on the utility of AS are generally in accordance with recent findings [13–16], the substrate-specific nature of CYP2D6 should be taken into consideration when interpreting the results.

5 Conclusions

Our results show that CYP2D6 metaboliser phenotype categories include patients with markedly different CYP2D6 activities, thereby causing inaccuracies in phenotype-based predictions. Using CYP2D6 activity scores as an ordinal continuous covariate instead of translating genetic makeup into metaboliser categories, we could harness the changes in metabolic characteristics and better account for the inter-individual variability. This approach shows the utility of *CYP2D6*-based AS covariates in model-informed codeine dosing in comparison with phenotype categories.

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Declarations

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Competing Interests This study was a non-commercial, investigator-initiated trial and has not received any funding from the industry. The authors have no financial or proprietary interests in any material discussed in this article.

Ethics Approval The study protocol was reviewed and approved by approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District (3/2016) and the Finnish National Agency for Medicines (KL no. 4/2016).

Consent to Participate All participants provided written informed consent before study participation.

Consent for Publication All authors approved this manuscript for publication.

Availability of Data Patient-level data for data on file in this manuscript are not available for sharing as there is a reasonable likelihood that individual patients could be re-identified. Further information can be requested from the authors.

Author Contributions S.P., K.T.O. and V.K. conceived the idea and designed the study, contributed to the interpretation of the manuscript and gave final approval of the version to be published; M.Ne, J.I.K, J.B. and M.Ni. contributed to data analyses, interpretation of the manuscript and gave final approval of the version to be published; M.W.A. and T.I.S. supported in study design, collected model information and performed simulations and wrote and finalised the manuscript.

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