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ARTICLE

Delineating gene–environment effects using virtual twins of patients treated with clozapine

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Abstract

Studies that focus on individual covariates, while ignoring their interactions, may not be adequate for model-informed precision dosing (MIPD) in any given patient. Genetic variations that influence protein synthesis should be studied in conjunction with environmental covariates, such as cigarette smoking. The aim of this study was to build virtual twins (VTs) of real patients receiving clozapine with interacting covariates related to genetics and environment and to delineate the impact of interacting covariates on predicted clozapine plasma concentrations. Clozapine-treated patients with schizophrenia ($N = 42$) with observed clozapine plasma concentrations, demographic, environmental, and genotype data were used to construct VTs in Simcyp. The effect of increased covariate virtualization was assessed by performing simulations under three conditions: "low" (demographic), "medium" (demographic and environmental interaction), and "high" (demographic and environmental/genotype interaction) covariate virtualization. Increasing covariate virtualization with interaction

improved the coefficient of variation (R^2) from 0.07 in the low model to 0.391 and 0.368 in the medium and high models, respectively. Whereas R^2 was similar between the medium and high models, the high covariate virtualization model had improved accuracy, with systematic bias of predicted clozapine plasma concentration improving from −138.48 ng/ml to −74.65 ng/ml. A high level of covariate virtualization (demographic, environmental, and genotype) may be required for MIPD using VTs.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Between-patient variability in clozapine pharmacokinetics (PKs) is common in clinical practice. Covariates affecting this variability in PKs include demographic (e.g., sex and age), genetic (e.g., CYP genotypes), and environmental (e.g., smoking status and drug interactions) factors.

WHAT QUESTION DID THIS STUDY ADDRESS?

Can systematically increasing the number of covariates ("virtualization") in physiologically-based pharmacokinetic (PBPK) modeling improve the prediction of clozapine plasma concentration using a virtual twin (VT) approach?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

The study demonstrated improvement in the prediction of clozapine PK by increasing the virtualization of VTs, and this serves as proof of concept that a high level of covariate virtualization is required for model-informed precision dosing (MIPD).

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

Incorporating gene–environment interactions in PBPK modeling can help improve the accuracy of model predictions and advance the field one step closer to realize the potential of MIPD in clinical practice.

INTRODUCTION

An individual demographic, genetic, or environmental covariate may not influence pharmacokinetics (PKs) significantly when studied in isolation. But when modeled collectively using interaction terms, the combined effect of covariates may be significant.[1,2] Modeling approaches that quantify these interactions between variables across a target population may be used to optimize starting and continued dosing, an important consideration for model-informed precision dosing (MIPD).[3] Physiologically-based pharmacokinetic (PBPK) modeling is well-suited for MIPD due to its highly mechanistic basis and the ability to consider concurrently the impact of patient, drug, and environmental variables on PKs. In recent years, validated PBPK platforms have been used to construct "virtual twins" (VTs) of real patients to predict PKs by incorporating a large number of variables, including ethnicity, sex, age, genotype/phenotype of drug metabolizing enzymes and transporters, and co-administered medications.[3–6] This approach, MIPD-VT, can rapidly delineate the impact on PK predictions when demographic, gene, and environmental covariates are considered individually or when they are considered together, analogous to an interaction term in other modeling approaches.

Clozapine is the only approved antipsychotic for treatment-resistant schizophrenia.[7] It has a narrow therapeutic index and potentially life-threatening adverse effects, including cardiomyopathy, agranulocytosis, and seizures. Precision dosing of clozapine via therapeutic drug monitoring (TDM) is essential to improve clozapine safety.[8] Although TDM naturally accounts for all interactions between genetic and environmental variables post-dosing, the associated models used to analyze the TDM data rarely use interaction terms in modeling the influence of various parameters. This stems from a lack of mechanistic insights into how these factors or their interaction influence clozapine PKs.[9] The outcome from such purely statistical models, implemented in current TDM software, cannot be used as a priori information to tailor the dose before commencing drug treatment and doing TDM. These also lead to misunderstanding the effects of parameters, or

lack thereof, because such effects could be observed only in a certain space associated with other parameters, such as age or comedication-dependent effects of genetics on clearance[10,11] or the metabolic drug-interaction which are dependent on renal function.[12]

To further understand the capacity of VTs to delineate combined gene–environment effects, clozapine was chosen for this study because many covariates are known to influence its PKs.[13] Indeed, clozapine is extensively metabolized by cytochrome P450 (CYP) 1A2 (CYP1A2), with CYP3A4, CYP2D6, CYP2C19, and CYP2C9 playing relatively minor roles.[14–16] This means that genetic variation and the presence of CYP inducers and inhibitors all contribute to variability in the phenotype of these CYPs. In particular, cigarette smoking status is a clinically actionable covariate that must be considered when dosing clozapine, because changes in the expression of CYP1A2, and subsequently clozapine clearance, strongly influence plasma concentrations.[13] Smokers carrying the inducible *CYP1A2*1F/*1F* genotype are at an increased risk of non-response to clozapine due to reduced plasma concentration.[17–19] Concomitant medications inhibiting CYP1A2 (e.g., ciprofloxacin and fluvoxamine) or inducing CYP1A2 (e.g., carbamazepine and phenytoin) also alter clozapine plasma concentrations and response, although the degree to which CYP1A2 genotype influences the severity of these drug–drug interactions (DDIs) is less well understood.

To further understand the implications of gene–environment interactions for MIPD, the aim of this study was to determine whether systematically increasing the number of modeled covariates ("virtualization") improves the prediction of clozapine plasma concentration using a VT approach. This work was conducted as a step forward for MIPD-VT. In other words, MIPD-VT could be used to predict optimal starting doses of clozapine, and then TDM used with standard dose proportional adjustments or Bayesian methods to nuance the dose.

METHODS

Participants

Forty-two patients with schizophrenia who were enrolled in a previous clinical study with clozapine were selected to construct VTs in Simcyp.[20] The internally validated drug profile for clozapine in Simcyp was then used to predict the observed clozapine trough concentrations (see Table 1). Participants with known CYP1A2 genotypes (*CYP1A2*1A/*1A*, **1A/*1F*, or **1F/*1F)* were selected to assess the importance of customizing the CYP1A2 enzyme abundance based on the inducible *CYP1A2*1F/*1F* genotype. Participants carrying CYP1A2 alleles with unknown function (e.g., **1L* or **1V*) were excluded. The concomitant inhibitors and inducers with potential to influence clozapine exposure were fluoxetine, citalopram, sertraline, clobazam, esomeprazole, pantoprazole, oral contraceptives, valproate, and cigarette smoking.[1] Ethical approval for the clinical study with clozapine was provided by the Melbourne Health Human Research Ethics Committee (MHREC ID 2012.069 and 2012.066) and complied with the Declaration of Helsinki and its subsequent revisions.[21]

Genotype testing and phenotype assignment

Details of sample collection, processing, and determination of genotype were described previously.[20] Briefly, blood samples were collected at steady-state ~12 h after the last clozapine dose to extract DNA for genotyping and measure clozapine plasma concentrations. The term "genotype" used in the current study refers to diplotypes of CYP1A2, CYP2C19, CYP2D6, CYP2C9, CYP3A4, and CYP3A5. These were determined using TaqMan-based assays by myDNA (myDNA; Life Australia Limited, Melbourne, Australia). Clozapine plasma concentrations were measured using liquid chromatograph tandem mass spectrometry. Genotype to phenotype translation was based on definitions provided by the Pharmacogene Variation Consortium[22] and the Clinical Pharmacogenetics Implementation Consortium (CPIC).[23] Genotype testing was also performed on unlinked ancestry-informative markers to assign each participant to one of the following populations: Northern/Western European, Han Chinese, or Yoruba in Nigeria.

Construction of VTs

Individual VTs were constructed in Simcyp by matching for each participant's demographics, clozapine dose, and presence of inhibitors and inducers (see Table 1). The demographic parameters of height, weight, and sex were fixed in each VT by changing the coefficient of variation (CV) to zero. The age was fixed by making the minimum and maximum age of the VT equivalent to the age of the study participant. The CVs for system components, such as hematocrit, tissue composition, and enzyme and transporter abundances, used the inbuilt values within Simcyp and were not fixed. Esomeprazole and fluoxetine were the only inhibitors with validated inhibitor drug profiles in Simcyp and were accounted for where appropriate. The daily dose of fluoxetine and esomeprazole was not recorded in the original study and therefore a dose of 40 mg

TABLE 1 Patient characteristics in the clinical study.

Age (yrs)	Ancestry	Sex	Clozapine dose	Smoking (C/D)	Alcohol intake	CYP1A2	CYP2D6	CYP2C19	CYP2C9	CYP3A4	CYP3A5	Ht (m)	Wt (kg)	BMI	Concomitant Inh/ind
37	E	M	100 mg Nocte	Non-smoker	Low	*1A/*1F	*1/*2	*2/*17	*1/*1	*1/*1	*3/*3	1.74	84	27.9	
32	E	M	175 mg Nocte	Non-smoker	Low	*1F/*1F	*1/*4	*2/*17	*1/*1	*1/*1	*3/*3	1.79	144	45.1	
35	E	F	200 mg Nocte	Non-smoker	Moderate	*1F/*1F	*3/*4	*1/*1	*2/*3	*1/*1	*3/*3	1.57	87	35.4	Citalopram
39	E	M	200 mg Nocte	Non-smoker	None	*1A/*1F	*2/*2	*1/*1	*1/*1	*1/*1	*3/*3	1.80	100	30.9	
34	0	F	200 mg Mane	Non-smoker	Low	*1A/*1F	*2/*4	*17/*17	*1/*1	*1/*1	*3/*3	1.60	64	24.9	Fluoxetine
33	0	M	200 mg Nocte	11–20	Low	*1A/*1A	*1/*4	*1/*1	*1/*2	*1/*1	*3/*3	1.91	100	27.4	
49	0	F	200 mg Nocte	Non-smoker	Low	*1A/*1F	*4/*4	*1/*1	*1/*2	*1/*1	*3/*3	1.60	46	18.1	Sertraline, oral contraceptive
22	E	M	250 mg Nocte	<10	Low	*1F/*1F	*1/*10	*1/*1	*1/*3	*1/*1	*3/*3	1.94	106	28.2	
35	E	M	250 mg Nocte	11–20	Moderate	*1F/*1F	*1/*2	*1/*2	*1/*1	*1/*1	*3/*3	1.73	97	32.4	
27	E	M	275 mg Nocte	21–30	None	*1F/*1F	*1/*2	*1/*1	*1/*2	*1/*1	*3/*3	1.70	61	21.4	Sodium valproate
27	E	M	300 mg Nocte	21–30	Low	*1A/*1F	*1/*4	*1/*1	*1/*2	*1/*22	*3/*3	1.73	117	39.2	Esomeprazole
51	E	M	300 mg Nocte	Non-smoker	High	*1A/*1A	*1/*2	*1/*2	*1/*3	*1/*1	*3/*3	1.89	90	25.1	
47	E	M	300 mg Nocte	Non-smoker	Low	*1A/*1F	*4/*41	*1/*1	*1/*1	*1/*1	*3/*3	1.68	78	27.6	
58	E	M	325 mg Nocte	Non-smoker	None	*1F/*1F	*2/*4	*1/*1	*1/*1	*1/*1	*3/*3	1.78	127	40.1	Citalopram, esomeprazole, sodium valproate
32	E	M	350 mg Nocte	Non-smoker	Low	*1A/*1F	*1/*4	*1/*2	*1/*1	*1/*1	*1/*3	1.87	135	38.6	Sertraline
45	E	M	375 mg Nocte	<10	Low	*1F/*1F	*2/*5	*1/*1	*1/*1	*1/*1	*3/*3	1.81	79	24.1	
24	0	M	75 mg midi, 300 mg nocte	11–20	None	*1A/*1A	*17/*17	*1/*1	*1/*1	*1/*1	*1/*1	1.87	71	20.4	
41	E	M	375 mg Nocte	Non-smoker	None	*1A/*1A	*4/*4	*1/*1	*1/*3	*1/*22	*3/*3	1.70	77	26.7	Fluoxetine, esomeprazole, sodium valproate
38	E	M	375 mg Nocte	Non-smoker	None	*1A/*1F	*1/*2	*1/*17	*1/*1	*1/*1	*1/*3	1.74	95	31.3	Esomeprazole
58	E	M	400 mg Nocte	Non-smoker	None	*1A/*1F	*2/*4	*1/*2	*1/*3	*1/*1	*3/*3	1.84	153	45.2	
45	E	F	400 mg Nocte	Non-smoker	Low	*1A/*1A	*1/*4	*1/*1	*1/*1	*1/*1	*3/*3	1.60	90	35.3	
45	0	F	200 mg mane, 200 mg nocte	11–20	Low	*1A/*1F	*1/*1	*1/*1	*1/*1	*1/*1	*3/*3	1.72	95	32.1	
55	A	F	425 mg Nocte	Non-smoker	Low	*1A/*1F	*1/*41	*1/*17	*1/*1	*1/*1	*3/*3	1.57	68	27.6	
39	0	M	425 mg Nocte	<10	Low	*1F/*1F	*1/*1	*1/*17	*1/*1	*1/*1	*1/*3	1.88	147	41.6	Sodium valproate

(Continues)

TABLE 1 (Continued)

Age (yrs)	Ancestry	Sex	Clozapine dose	Smoking (C/D)	Alcohol intake	CYP1A2	CYP2D6	CYP2C19	CYP2C9	CYP3A4	CYP3A5	Ht (m)	Wt (kg)	BMI	Concomitant Inh/ind
33	0	F	425 mg Nocte	<10	Low	*1F/*1F	*1/*1	*1/*17	*1/*3	*1/*1	*3/*3	1.68	85	30.1	
44	E	M	450 mg Nocte	Non-smoker	None	*1A/*1F	*1/*3	*1/*2	*1/*1	*1/*1	*3/*3	1.72	73	24.8	Sodium valproate
37	E	M	500 mg mane	21–30	None	*1A/*1A	*10/*41	*1/*17	*1/*3	*1/*1	*1/*3	1.89	104	29.1	
37	E	M	500 mg Nocte	11–20	None	*1F/*1F	*1/*1	*1/*17	*1/*1	*1/*1	*3/*3	1.76	123	39.6	
37	E	M	500 mg Nocte	Non-smoker	None	*1F/*1F	*1/*1	*1/*1	*2/*2	*1/*22	*3/*3	1.74	94	31.2	Sertraline
47	0	F	500 mg Nocte	Non-smoker	Low	*1A/*1F	*1/*9	*1/*17	*1/*1	*1/*1	*3/*3	1.72	77	26.1	Citalopram, esomeprazole
49	E	F	525 mg Nocte	11–20	None	*1F/*1F	*4/*6	*1/*1	*1/*1	*1/*1	*1/*3	1.61	107	41.3	Citalopram
41	E	M	550 mg (400 mg nocte, 150 mg mane)	21–30	Low	*1F/*1F	*1/*2	*2/*2	*1/*1	*1/*1	*3/*3	1.73	89	29.7	
39	0	F	550 mg Nocte	Non-smoker	None	*1A/*1A	*1/*9	*1/*1	*1/*1	*1/*1	*3/*3	1.58	65	26.1	Citalopram
36	E	M	575 mg Nocte	Non-smoker	None	*1F/*1F	*2/*4	*1/*1	*1/*1	*1/*1	*3/*3	1.77	85	27.2	
34	E	M	600 mg Nocte	21–30	Moderate	*1F/*1F	*1/*1	*1/*17	*1/*1	*1/*1	*3/*3	1.76	159	51.3	
22	E	M	600 mg Nocte	11–20	Moderate	*1F/*1F	*4/*4	*1/*17	*1/*2	*1/*1	*3/*3	1.71	73	25.2	Citalopram
30	0	F	600 mg (100 mg mane, 100 mg midi, 400 mg nocte)	Non-smoker	None	*1A/*1F	*1/*41	*2/*17	*1/*1	*1/*1	*1/*3	1.66	124	44.7	Fluoxetine, pantoprazole
40	0	M	600 mg (50 mg mane, 550 mg nocte)	11–20	Low	*1A/*1F	*2/*2	*17/*17	*1/*1	*1/*1	*3/*3	1.73	94	31.4	
54	E	M	650 mg Nocte	21–30	None	*1A/*1A	*1/*2	*2/*17	*1/*1	*1/*1	*3/*3	1.85	154	45.0	Pantoprazole
53	E	M	650 mg (150 mg mane, 500 mg nocte)	Non-smoker	None	*1A/*1A	*1/*1	*2/*2	*1/*1	*1/*1	*3/*3	1.81	80	24.4	Fluoxetine
45	E	M	675 mg Nocte	21–30	Low	*1A/*1F	*1/*1	*1/*1	*1/*1	*1/*1	*1/*3	1.70	86	29.6	
49	E	M	900 mg Nocte	>31	None	*1F/*1F	*1/*1	*1/*1	*2/*2	*1/*1	*3/*3	1.92	96	26.2	Esomeprazole

Abbreviations: A, Han Chinese; C/D, Cigarettes per Day; E, Northern and Western European; F, Female; Ht, Height; Ind, Inducer; Inh, Inhibitor; M, Male; O, Other; Wt, Weight.

daily was assumed for each drug based on recommended dosage ranges used in clinical practice.[24–26]

The Simcyp population of healthy volunteers was used for participants with a body mass index (BMI) of less than 30 and assigned as having Northern and Western European or "other" ancestry, as per demographic data in Table 1. The obese population in Simcyp was used for participants with a BMI between 30 and 40, whereas the morbidly obese population was used for those with a BMI greater than 40, irrespective of ancestry. The Simcyp population of Chinese healthy volunteers was used for one participant who was assigned as having Asian ancestry with a BMI less than 30.

Cytochrome P450 phenotypes are defined by enzyme abundance (pmol mg^{-1} microsomal protein) and the turnover rate constant (1 h^{-1}) in Simcyp. Smokers with a *CYP1A2*1F/*1F* genotype were assigned a CYP1A2 enzyme abundance based on the number of cigarettes smoked per day, as described previously by Plowchalk et al.[27] In short, enzyme abundance was individually adjusted based on the number of cigarettes smoked per day, using the following groupings: <10 cigarettes/day (conservatively used 64 pmol mg^{-1} protein), 11–20 cigarettes/day (90 pmol mg^{-1} protein), and >20 cigarettes/day (94 pmol mg^{-1} protein). One participant consumed more than 30 cigarettes per day and a CYP1A2 enzyme abundance of 156 pmol mg^{-1} protein was used.[28] All other participants were assigned a nonsmoker CYP1A2 enzyme abundance of 52 pmol mg^{-1} microsomal protein. Smokers with the *CYP1A2*1A/*1A* or *CYP1A2*1A/*1F* genotype were assumed to have minimal or no induction by cigarette smoking and therefore were assigned the nonsmoker CYP1A2 enzyme abundance.

Simcyp provides CYP2D6, CYP2C19, and CYP2C9 enzyme abundance data for the following phenotypes (CYP2D6 and CYP2C19 – poor metabolizer [PM], normal metabolizer [NM also known as EM], and ultrarapid metabolizer [UM]; CYP2C9 – PM and NM). Intermediate metabolizer phenotypes were assigned enzyme abundances based on the following algorithm provided by the Simcyp product team (Simcyp; Certara, personal communication). For CYP2D6, CYP2C19, and CYP2C9, the NM enzyme abundance was respectively multiplied by a factor of 0.362, 0.646, and 0.876. Participants with the *CYP3A4*1/*22* genotype ($N = 3$) were treated as NMs in Simcyp, as there is variability surrounding the available data for the *CYP3A4*22* allele and it is thought that this allele's overall activity in the wider population is undistinguishable from an NM. Carriers of the *CYP3A5*1/*3* genotype were also treated as NMs as CYP3A5 is thought to play a minor role in the metabolism of clozapine and the corresponding enzyme abundance is not available.

Stabilization of VT estimates

All simulations were performed using Simcyp version 19 (Certara, Princeton, NJ). To optimize the model's prediction and get a stable median plasma trough clozapine concentration for each VT, simulations for two participants were run for 14 days at trials of 10, 100, 1000, and 5000 to see which best captures the true and stable clozapine distribution based on the central limit theorem.

VT predictions

To assess the effect on predicting plasma clozapine trough concentrations by increasing covariate virtualization, simulations were performed under the following conditions:

(i) Low covariate virtualization (demographic) – VTs were matched for height, weight, age, and clozapine dose only. Patient-specific CYP phenotypes and concomitant inhibitors and inducers were omitted from the model. The Simcyp inbuilt population data was used to inform CYP phenotypes and all other system parameters.

(ii) Medium covariate virtualization (demographic and environmental) – VTs were matched for height, weight, age, clozapine dose, and concomitant CYP inducers and inhibitors. CYP1A2 enzyme abundance was matched to the number of cigarettes smoked per day based on published figures described above. Genotype data was omitted from the model and Simcyp inbuilt population phenotype data was used to inform CYP phenotypes and other system parameters.

(iii) High covariate virtualization (demographic, environmental, and genotype) – VTs were matched for height, weight, age, clozapine dose, inducers, inhibitors, and CYP genotype-predicted phenotypes for CYP2D6, CYP2C19, and CYP2C9. The CYP1A2 enzyme abundance values were matched to the number of cigarettes smoked per day for *CYP1A2*1F/*1F* carriers only. All other CYP1A2 genotypes (**1A/*1F* and **1A/*1A*) were assigned the enzyme abundance value for nonsmokers (52 pmol mg^{-1} microsomal protein).

Statistical analysis

GraphPad Prism (version 8.0.0 for Windows; GraphPad Software, San Diego, CA, www.graphpad.com) was used to perform descriptive analysis and produce the Bland–Altman plots and the simple linear regressions graphs.

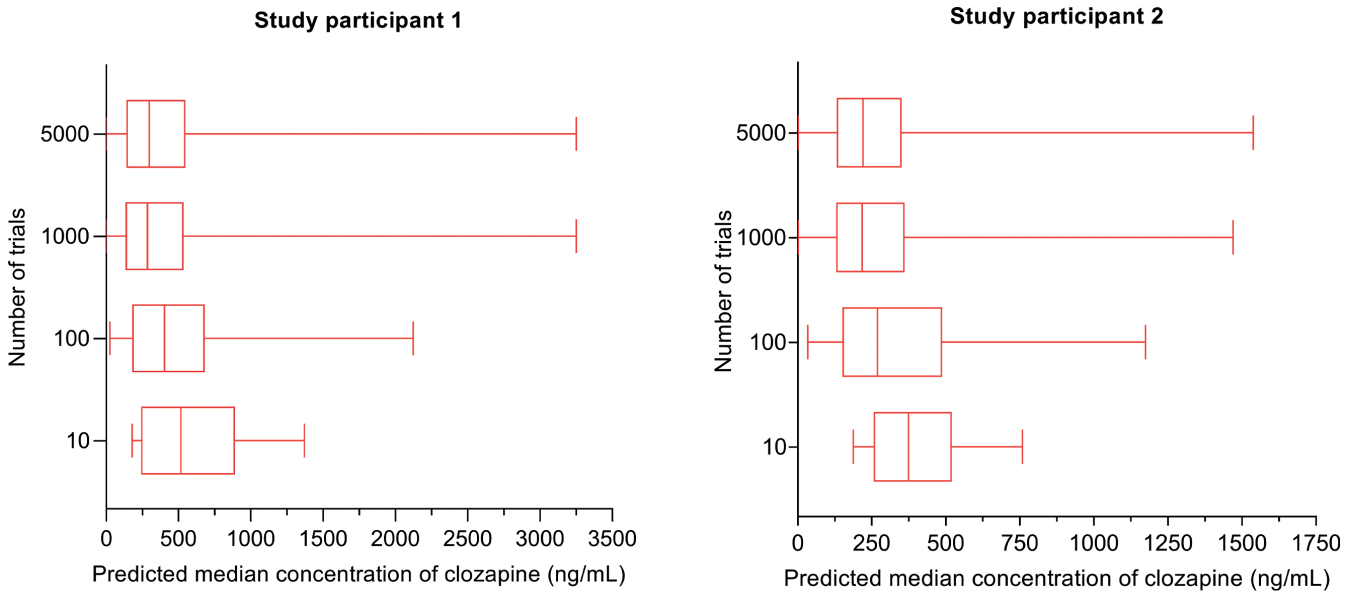

FIGURE 1 Clozapine median concentrations of two study participants at simulations of 10, 100, 1000, and 5000 trials

RESULTS

Stabilization of VT estimates

Simulations using inputs from two study participants showed that 1000 trials per VT captured a stable median for clozapine trough concentration. Further increases in trial number did not yield any significant variation in the median concentration of clozapine, as shown in the box and whisker plots of Figure 1.

VT predictions

Figure 2 shows predicted versus observed trough clozapine concentrations for the full cohort ($N = 42$) in three categories with increasing covariate virtualization (Figure 2a–c). The corresponding Bland–Altman plots are provided in Figure 2d–f.

Low covariate virtualization

Figure 2a shows the observed versus predicted plasma clozapine plasma trough concentrations for the full cohort where the following data was omitted (CYP1A2, CYP2D6, CYP2C19, and CYP2C9 genotype predicted phenotypes, co-administered inhibitors, and inducers, including cigarette smoking). There was a relatively poor relationship between observed versus predicted plasma clozapine trough concentrations with a correlation of determination (R^2) value of 0.07. The corresponding Bland–Altman plot (Figure 2d) demonstrates a systematic bias resulting in underprediction of the plasma trough clozapine concentration (−35 ng/ml) with a bias SD of 271.7 ng/ml.

Medium covariate virtualization

Figure 2b shows an improved relationship and precision in the prediction of plasma clozapine trough concentrations (R^2 of 0.391) when CYP1A2 induction by cigarette smoking and concomitant inhibitors were accounted for in the model. The corresponding Bland–Altman plot (Figure 2e) suggests a systematic bias resulting in underprediction of the plasma trough clozapine concentration (−138.48 ng/ml) with a reduced bias (SD of 200.6 ng/ml).

High covariate virtualization

Figure 2c presents the VT model matching for inhibitors, CYP1A2, CYP2D6, CYP2C19, and CYP2C9 genotype predicted phenotypes, CYP1A2 abundance based on the *CYP1A2*1F/*1F* genotype, and the number of cigarettes smoked per day. This model produced a similar correlation to the model in Figure 2b with an R^2 value of 0.368, however, the confidence interval of the line of best fit included the line of unity. Furthermore, the corresponding Bland–Altman plot (Figure 2f) suggests an improved prediction of plasma trough clozapine concentration with an underprediction systematic bias (−74.65 ng/ml) and a bias SD of 206.8 ng/ml, similar to the medium covariate virtualization model (Figure 2e).

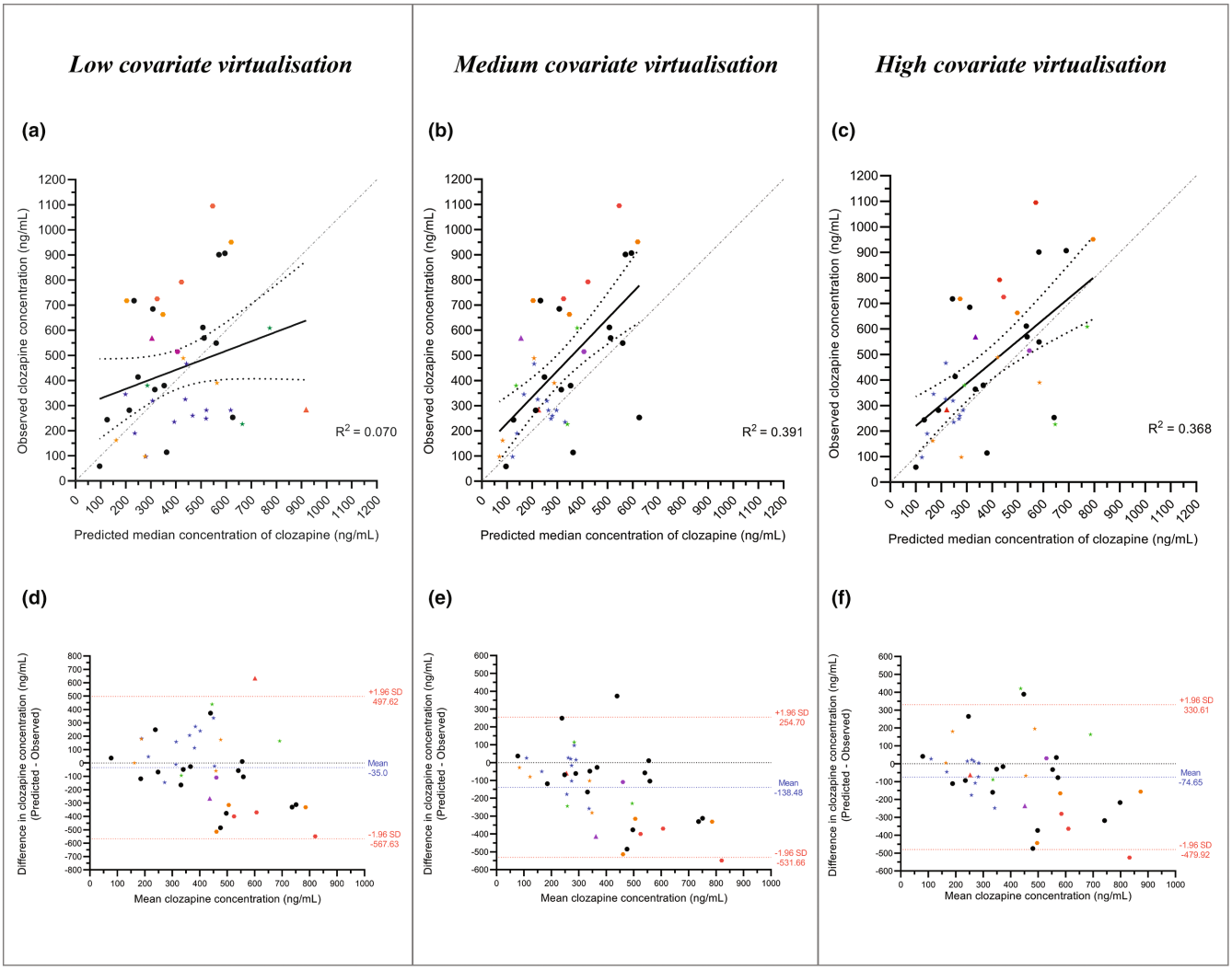

FIGURE 2 Linear regression plots showing predicted vs observed clozapine systemic median trough concentrations. (a) VTs matched for weight, height, age, and clozapine dose only; (b) VTs matched for weight, height, age, clozapine dose, inducers, and inhibitors. Phenotypes were maintained at population frequencies and CYP1A2 enzyme abundance matched to the number of cigarettes smoked per day irrespective of CYP1A2 genotype. (c) VTs matched for weight, height, age, clozapine dose, inducers, inhibitors, and CYP phenotypes. Participants with the *CYP1A2*1F/*1F* genotype had enzyme abundances matched to the number of cigarettes smoked per day and others were assigned the enzyme abundance of nonsmokers (52 pmol mg^{-1} microsomal protein). The corresponding Bland–Altman plots are shown in (d), (e), and (f). ● Nonsmokers, ★ smokers (*CYP1A2*1A/*1A*), ★ smokers (*CYP1A2 *1A/*1F*), ★ smokers (*CYP1A2*1F/*1F*), ▲ smoker (esomeprazole and *CYP1A2*1F/*1F*), ▲ smoker (esomeprazole and *CYP1A2*1A/*1F*), ⬢ nonsmoker (fluoxetine), ⬢ nonsmoker (esomeprazole), and ⬢ nonsmoker (esomeprazole and fluoxetine). The solid line on the graphs represents the line of best fit. The dotted lines represent the 95% confidence interval of the slope and the dashed lines represent the line of unity. VT, virtual twin.

DISCUSSION

Many demographic, genetic, and environmental factors can impact PKs, however, when studied in isolation, individual covariates may not appear to play a major role. However, the combined effect of covariates may be significant and require modeling using interaction terms or by including multiple essential covariates in PBPK-based simulations. Three PBPK models with varying degrees of covariate virtualization (low, medium, and high) were assessed to understand the impact of gene–environment interactions on the accuracy of VT predictions. The key finding was that VT predictions can be improved by increasing the covariate virtualization through incorporation of demographic, genetic, and environmental data. The high covariate virtualization model produced the best prediction of plasma clozapine trough concentration compared to the low and medium covariate level virtualization models.

Three previous studies have used VTs/PBPK modeling to predict plasma trough clozapine concentrations. Ghoneim et al.[29] demonstrated adequate predictions in

a healthy adult population, although the modeling approach was not validated in patients. Lee et al.[30] developed a clozapine PBPK model that was optimized for Korean patients, demonstrating that age and sex were important covariates for clozapine clearance. However, this model did not assess genetic or environmental factors known to alter clozapine exposure. More recently, Wills et al.[31] used PBPK modeling to assess the contribution of physiological covariates to the degree of variability in clozapine exposure in patients. The physiological covariates of sex and CYP1A2 abundance were confirmed as significantly associated with clozapine concentrations. Modeling, however, underperformed compared to TDM in cases where environmental factors, such as altered adherence and drug interactions, were the main source of variability.

It has been previously suggested that dose, sex, age, and smoking account for ~50% of the variability in plasma clozapine concentration in patients, whereas the other 50% is accounted for by genetic variations in CYP enzymes and drug interactions.[32,33] To depict the real-world effects encountered in patients prescribed clozapine and address the perceived limitations of previous clozapine PBPK models, we optimized a PBPK model to account for genotype, concomitant drugs, sex, age, ancestry, and BMI to test the impact of enhanced virtualization on model performance. Our high covariate virtualization model resulted in an overall improvement in the accuracy of model predictions with an underprediction systematic bias of −74.65 ng/ml compared to −138.48 ng/ml in the medium covariate virtualization model. Indeed, our approach of customizing the CYP1A2, CYP2D6, CYP2C19, and CYP2C9 enzyme abundances based on genotype improved plasma clozapine trough concentration predictions. Figure 2a–c allow visualization of the impact each added covariate has on the prediction of clozapine plasma concentration. Importantly, the increased virtualization via the addition of genotype data shown in Figure 2c produced the most improved predictions and the majority of smoker and nonsmoker groups (as defined in the legend of Figure 2) moved closer to the line of unity.

Our genotype guided approach in customizing the enzyme abundances for CYP1A2, CYP2D6, CYP2C19, and CYP2C9 in the high covariate virtualization and interaction model was a key differentiator in comparison to the medium covariate virtualization model. The CYP1A2 enzyme abundance was selected as a function of genotype and the number of cigarettes smoked per day. This approach is supported by previous studies which demonstrated higher enzyme induction and lower clozapine plasma concentration in smokers homozygous for the *CYP1A2*1F (−163 C>A* variant) allele.[19,34–37] To ensure the CYP1A2 genotype induction effect was captured accurately, carriers of other CYP1A2 haplotypes containing the CYP1A2 variant (*−163 C>A*) were excluded as their function is currently unknown (*CYP1A2*1L* and *CYP1A2*1V*).[38] The customization of CYP2D6, CYP2C19, and CYP2C9 enzyme abundances was based on genotype and also contributed to improved model performance, including cases with drug interactions by fluoxetine and esomeprazole, further emphasizing the importance of the combined gene–environment effect. Despite the overall underprediction of the high covariate virtualization model in subjects (smokers and nonsmokers) taking esomeprazole and fluoxetine, the model still produced predictions closer to the observed concentrations, as shown in Figure 2b,c. Model underprediction of fluoxetine DDIs has been reported in previous studies.[11,39] Apart from its CYP2D6 strong inhibition, fluoxetine is also metabolized by CYP2D6 and variability in CYP2D6 clearance of fluoxetine has been suggested as a possible explanation for this observed underprediction. We propose a similar explanation can be applied to esomeprazole as it is metabolized by CYP2C19 which it also moderately inhibits. Our combined demographic, genetic, and environmental covariate interaction (CYP genotypes, medications, and smoking, respectively) improved the overall performance of the model. A 40 mg daily dose for esomeprazole and fluoxetine was used for simulations despite the actual doses not recorded in the clinical dataset. Because a 20 mg dose of each medication is also used clinically, additional simulations were performed at this dose but they showed no significant differences in predicted clozapine concentrations compared with the higher dose (data not shown).

We acknowledge there is a lack of consensus in the literature regarding the influence of *CYP1A2*1F* on the inducibility of the CYP1A2 enzyme in cigarette smokers. Some studies have failed to show an induction effect from *CYP1A2*1F*, whereas others have demonstrated increased CYP1A2 inducibility and lower clozapine plasma concentration specifically in homozygous *CYP1A2*1F* smokers.[17,19,34] In our study, we assumed increased induction only in homozygous *CYP1A2*1F* smokers and recognize this to be a potential limitation due to this lack of consensus in the literature. Another limitation of the study is that some concomitant drugs known to be CYP inhibitors and inducers could not be modeled because validated compound files were unavailable. Indeed, there were several weak and moderate inhibitors of clozapine metabolism listed by the study participants (e.g., citalopram, sertraline, clobazam, pantoprazole, oral contraceptives, and valproate), however, simulations were limited to the inclusion of only fluoxetine and esomeprazole because they have validated compound files in Simcyp. As the compound files in PBPK M&S platforms become more comprehensive, the impact of all CYP inhibitors and inducers can be further investigated and evaluated, thus further increasing virtualization. Another limitation is that

the exact timing of blood sample collection and the last clozapine dose prior to blood sampling were not available. Based on the standard clozapine protocol it was assumed that blood samples taken for measurement of clozapine concentration were collected 12 h after the last dose. Simulated plasma clozapine concentrations were taken at exactly 12 h after the last dose. We acknowledge that blood samples taken before or after 12 h from the last clozapine dose could be a source of inaccuracy in predictions.

Importantly, we have found that performing 1000 trials per simulation produced a stable mean clozapine concentration. Increasing the number of trials above 1000 was not necessary and produced a minimal change in the mean clozapine plasma concentration. This approach may be considered for simulations of other drugs using a similar MIPD-VT method.

The PBPK platforms are primarily utilized for drug development and in the design of DDI studies. In recent years, PBPK models have been effective at describing drug interactions with perpetrator drugs and gene-DDIs.[11,39] With the further utilization of pharmacogenomics in clinical practice, there is increasing importance of recognizing and addressing gene-DDIs, which is also sometimes referred to as phenoconversion (a process where medications alter the genotype-predicted phenotype of a drug metabolizing enzyme).[40,41] The future implementation of PBPK-based MIPD into routine clinical care is expected to help address the complexities of phenoconversion. Importantly, a level of optimization of PBPK models is necessary for the clinical environment. We therefore suggest that a more comprehensive list of validated inhibitor and inducer compound files is required to cater for phenoconversion, including weak and moderate inhibitors and inducers. It is well-recognized that psychiatric patients on polypharmacy who are at risk of significant drug interactions are key candidates for the application of MIPD, especially with the projected increase in pharmacogenomic testing in these patients.[42] The present study demonstrates that by incorporating gene–environment interactions in PBPK modeling, we advance one step closer to realize the potential of MIPD in clinical practice. As a next logical step, this approach can be expanded through a time series analysis of clozapine TDM data points to assess model performance over time.

In conclusion, this study shows for the first time that increasing the virtualization of VTs improves the prediction of clozapine PKs, and supports the idea that a high level of covariate virtualization may be required for MIPD-VT.

AUTHOR CONTRIBUTIONS

S.M., T.M.P., C.M.J.K., A.R.-H., and L.J.S. wrote the manuscript. S.M., T.M.P., and C.M.J.K. designed the research. S.M. performed the research. S.M., T.M.P., and C.M.J.K. analyzed the data.

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CONFLICT OF INTEREST

S.M. and L.S. are employees and shareholders of myDNA Inc., a pharmacogenomic testing and interpretation company. T.M.P. provides a consultancy service to Sonic Genetics for the interpretation of pharmacogenomic test results. T.M.P. and A.R.-H. are employees of Certara, a company that provides modeling and simulation software and services to the pharmaceutical industry, including a population-based PBPK simulator (Simcyp). C.M.J.K. was the academic lead on the Certara-Monash Fellowship program co-funded by MTPConnect. C.A.B. is founder and equity holder of Sequence2Script Inc. and a member of the Clinical Pharmacogenetics Implementation Consortium and the Genetic Testing Committee of the International Society of Psychiatric Genetics. He has also received material support from Assurex, CNSDose, Genomind, and AB-Biotics for research purposes and has ongoing research collaborations with MyDNA but does not have equity, stocks, or options in these companies or any other pharmacogenetic companies. C.P. has participated on Advisory Boards for Janssen-Cilag, Astra-Zeneca, Lundbeck, and Servier. He has received honoraria for talks presented at educational meetings organized by Astra-Zeneca, Janssen-Cilag, Eli-Lilly, Pfizer,

Lundbeck, and Shire. All other authors declared no competing interests for this work.

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