

ORIGINAL ARTICLE

Dose Optimization of Efavirenz Based on Individual CYP2B6 Polymorphisms in Chinese Patients Positive for HIV

KH Hui¹, SS Lee^{2,3} and TN Lam^{1*}

The purpose of this study was to investigate the impact of CYP2B6-G516T polymorphisms on the pharmacokinetics (PKs) of efavirenz among the Chinese population and to propose doses for different genotypic populations that optimize therapeutic outcomes. Nonlinear mixed-effect modeling was applied to describe PKs of efavirenz in Chinese patients with human immunodeficiency virus (HIV). Probabilities of successful treatment at different doses were obtained by simulations using the developed model to identify the optimal doses. The model was based on data from 163 individuals. Efavirenz clearance was found to be significantly influenced by CYP2B6-G516T polymorphisms and body weight. The typical values of oral clearance were 10.2 L/h, 7.33 L/h, and 2.38 L/h and simulation results suggested that the optimal daily oral doses are 550 mg, 350 mg, and 100 mg for the GG, GT, and TT populations, respectively. The effect of CYP2B6-G516T polymorphisms on efavirenz clearance was successfully quantified. Pharmacogenetics-based dose individualization of efavirenz may optimize patient outcomes by promoting efficacy while minimizing central nervous system (CNS) side effects.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC? Efavirenz is known to have a narrow therapeutic window as well as a large interindividual variability in plasma levels. CYP2B6-G516T polymorphisms have been found to contribute to such variability. • WHAT QUESTION DID THIS STUDY ADDRESS? This study developed a population PK model of efavirenz and, hence, provided evidence for genotype-based dose individualization among the Chinese HIV-positive population. • WHAT THIS STUDY ADDS TO OUR KNOWLEDGE The study provides a Chinese-population specific PK model of efavirenz, demonstrates the potential of dose individualization to improve its clinical outcomes, and suggests optimal doses for different genotypes accordingly. Typical values of oral clearance are 10.2 L/h, 7.33 L/h, and 2.38 L/h and suggested optimal daily oral doses are 550 mg, 350 mg, and 100 mg for the GG, GT, and TT populations, respectively. • HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS The results of the study form the basis of clinical trials investigating the effects of genotype-based dose individualization of efavirenz; clinicians may prescribe an individualized dose for each genotype, leading to an increased rate of successful treatment of HIV-1 infection.

Efavirenz is a non-nucleoside reverse transcriptase inhibitor, which has played an important role as a component of the first-line regimen in highly active antiretroviral therapy used to treat type I human immunodeficiency virus (HIV-1) infection.^{1,2} Nevertheless, efavirenz is known to have a narrow therapeutic range. Efavirenz steady-state plasma levels (C_p) of <1 mg/L have been shown to be associated with insufficient viral suppression and, hence, treatment failure,^{3,4} whereas C_p of >4 mg/L are associated with central nervous system (CNS) side effects,³ such as insomnia and nightmares. This gave rise to a widely recommended therapeutic range of C_p of 1–4 mg/L. Unfortunately, there has been much observed variability in C_p among patients receiving the current, universally recommended efavirenz dose of 600 mg orally daily, resulting in, mainly, elevated C_p and, in turn, intolerable CNS side effects in many patients.

The large interindividual variability in the activity of cytochrome P450 2B6 (CYP2B6), the predominant isozyme metabolizing efavirenz, has been shown to contribute to the variability in C_p .⁵ Several studies have shown that the single nucleotide polymorphism of the CYP2B6 gene at codon 516 is associated with various C_p in HIV-1 infected patients—the T allele is associated with elevated C_p when compared to the G allele. It follows that the wildtype GG is associated with the lowest C_p , followed by the genotype GT and then the homozygous variant TT, which is associated with the highest C_p , usually above the therapeutic range.^{5–9} This is particularly relevant in populations with higher rates of the variant T allele, such as the Chinese (43%).¹⁰

Recently, the ENCORE1 study¹¹ and a study from Taiwan¹² provided support for universal efavirenz dose reduction and dose reduction according to the results of

¹School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong; ²Stanley Ho Centre for Emerging Infectious Diseases, Shatin, New Territories, Hong Kong; ³Department of Microbiology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong. *Correspondence: TN Lam (teddylam@cuhk.edu.hk)

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therapeutic drug monitoring, yet we doubt whether these strategies are appropriate. We hypothesized that reduced dosing for CYP2B6-G516T GT and TT genotypes has a greater potential to raise the rate of successful treatment than the above dosing strategies. In light of this, we conducted this project with two main purposes. First, we sought to develop a population pharmacokinetic (PK) model for efavirenz for the Chinese population linking CYP2B6-G516T polymorphisms to clearance (CL) of efavirenz. Then, we carried out PK and pharmacodynamic (PD) simulations with the developed model to find the optimal efavirenz dose for patients with each of the three CYP2B6-G516T polymorphisms (GG, GT, or TT) that would result in the largest proportion of individuals achieving successful treatment outcomes.

METHODS

Study population, data sources, and management of incomplete records

Retrospective clinical data of 79 subjects were collected from local routine therapeutic drug monitoring services of multiple HIV outpatient clinics (source #1). Data from three selected local prospective PK and clinical studies were also included to provide more intensive sampling data (source #2) and longitudinal data (sources #3 and #4) (see the **Supplementary Table S1** for the details of the data sources).^{13–15} The data items collected included dose, sampling times, CYP2B6-G516T genotype, C_p , sex, age, body weight and height, and co-medications when available. Missing body weights and heights were replaced by the mean values. In addition, records of times of sampling were not available for sources #1 and #4. Because patients were instructed to administer efavirenz at night time and blood samplings were taken early in the morning, sampling times for these subjects were assumed to be 12 hours postdose. The analyses included all Chinese adult patients taking efavirenz as part of their highly active antiretroviral therapy regimens. To eliminate the potential effects of incomplete enzyme autoinduction, samples collected within 7 days of the initiation of efavirenz treatment were excluded. The Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee provided the approval for the collection of data from previous studies.

Measurement of efavirenz plasma levels and genotyping

All C_p were measured using high performance liquid chromatography using stored blood (limit of quantification = 0.1 mg/L), as described by Notari *et al.*¹⁶ Genomic DNA was extracted from each blood sample using QIAmp DNA blood kits (Qiagen). All single nucleotide polymorphism of CYP2B6-G516T was detected by real-time polymerase chain reaction and TaqMan. Single nucleotide polymorphism genotyping assays were analyzed using the ABI 7500 sequence detection system (Applied Biosystems), as applied in a previous study by Xu *et al.*¹⁷ A CYP2B6-G516T genotype for each patient was identified as GG, GT, or TT.

The PK model

As assessed and applied in previous studies,^{18,19} a one-compartment model with first-order absorption was found to be appropriate for characterizing the PKs of efavirenz, therefore, it was used as the minimum structural PK model. Because the oral bioavailability (F) of efavirenz was not determined, CL and V_d represented apparent values (i.e., CL/F and V_d/F , respectively). We pre-estimated k_a from nine subjects from source #2 from whom intensive sampling during the absorption phase was available, and k_a was then fixed at the pre-estimated value in subsequent model development.

Demographic and genotypic covariates analyses

For demographic covariate analyses, sex, age, body weight, body height, and co-medications were assessed as potential covariates on CL and V_d . The relationship between demographic covariates and CL was investigated using the linear model, as well as for V_d (see **Table 1**, layer 1).

Before the genotypic covariate analyses, CYP2B6-G516T genotypes were assigned functional scores, according to the expected order of levels of enzymatic activity (i.e., 2, 1, and 0 for the CYP2B6-G516T genotypes of GG, GT, and TT, respectively).^{14,20} The relationship between CYP2B6-G516T genotypes and CL was explored with the use of the linear, square root, and logarithmic functions (see **Table 1**, layer 2). Both additive and proportional changes were tested.

Furthermore, because data were collected from the databases of different sources, we performed a preliminary two-way analysis of variance, including both genotype and data source as factors to identify any significant differences in C_p between the sources. In case of a positive finding, we would introduce data source as a covariate during model development to account for unexplained biases between the sources.

Unexplained variability analyses

A proportional error model (mean zero and variance ω^2) was applied to explain interindividual variability in CL . V_d was first fixed to a single population estimate; an error model was added later in model development. A proportional error model (mean zero and variance σ^2) was used for the description of intraindividual variability. Alternative error models, including the additive model and the exponential model, would be examined at a later stage of model development.

Estimation method, comparison between models and model evaluation

NONMEM version 7.2.0 (Icon PLC) was used to develop the population PK model. Initial estimates for fixed and random effects parameters were obtained or estimated from previously reported figures.^{13,18} The method of estimation used was the first order of conditional estimation with interaction. For model comparison, a ΔOF of 3.84 for each additional parameter indicated statistical significance at $\alpha = 0.05$ of the difference in goodness-of-fit between the two models. Backward elimination was performed at $\alpha = 0.01$ (i.e., ΔOF of 6.63) to identify any covariate added into the

Table 1 Details of model development

Layer	Models	CL	V _d	θ ₁	θ ₂	θ ₃	ΔOF	Sig
0	Minimum model	9.00	182					
1	$CL = \widehat{CL} \times [1 + \theta_1 \times (AGE - \mu_{AGE})]$	9.01	183	0.0035			-1.100	
	$CL = \widehat{CL} \times (1 + \theta_1 \times SEX)$	9.23	179	-0.181			-3.225	
	$CL = \widehat{CL} \times \{1 + \theta_1 \times [(WT/\mu_{WT}) - 1]\}$	8.99	183	0.535			-5.632	*
	$CL = \widehat{CL} \times [1 + \theta_1 \times (HT - \mu_{HT})]$	9.01	183	-0.0034			-0.248	
	$V_d = \widehat{V}_d \times \{1 + \theta_1 \times [(AGE/\mu_{AGE}) - 1]\}$	8.97	203	0.582			-0.663	
	$V_d = \widehat{V}_d \times (1 + \theta_1 \times SEX)$	8.93	221	-0.241			-0.683	
	$V_d = \widehat{V}_d \times \{1 + \theta_1 \times [(WT/\mu_{WT}) - 1]\}$	8.99	148	-1.29			-2.587	
	$V_d = \widehat{V}_d \times \{1 + \theta_1 \times [(HT/\mu_{HT}) - 1]\}$	8.95	194	5.26			-1.992	
2	$CL = \widehat{CL}_0 \times (1 + \theta_2 \times g)$	2.62	183	0.503	1.65		-110.037	**
	$CL = \widehat{CL}_0 \times (1 + \theta_2 \times \sqrt{g})$	2.38	180	0.54	2.34		-118.195	**
	$CL = \widehat{CL}_0 \times [1 + \theta_2 \times \ln(g+1)]$	2.42	180	0.529	3.07		-119.560	**
	$CL = I_{TT} \widehat{CL}_0 + I_{GT} \theta_2 + I_{GG} \theta_3$	2.41	180	0.531	7.64	10.5	-119.636	**
3	$CL = \widehat{CL} \times (1 + I_i \theta_3)$							
	→ Source #1 (i=1)	2.47	175	0.56	3.13	-0.0666	-1.610	
	→ Source #2 (i=2)	2.54	168	0.396	2.94	-0.168	-4.108	*
	→ Source #3 (i=3)	2.36	173	0.518	3.08	0.0524	-0.851	
	→ Source #4 (i=4)	2.41	184	0.492	3	0.465	-11.903	**
	$C_p = \widehat{C}_p \times (1 + I_i \theta_3)$							
	→ Source #1 (i=1)	2.47	175	0.558	3.12	0.0702	-1.563	
	→ Source #2 (i=2)	2.53	197	0.399	2.94	0.199	-4.421	*
	→ Source #3 (i=3)	2.37	173	0.519	3.08	-0.0468	-0.856	
	→ Source #4 (i=4)	2.41	183	0.494	3	-0.322	-12.054	**

CL, the apparent clearance of efavirenz; \widehat{CL} , the typical value of CL; \widehat{CL}_0 , the typical value of CL among TT subjects; C_p , the steady state plasma levels of efavirenz; \widehat{C}_p , the predicted value of C_p ; g , the functional score of the CYP2B6-G516T polymorphism ($g = 0, 1, \text{ and } 2$ for genotypes of TT, GT, and GG, respectively); I_{TT} , I_{GT} , and I_{GG} , the genotype indicator variable, where $I = 1$ if the individual has the indicated CYP2B6-G516T polymorphism, otherwise $I = 0$; I_i , the source indicator variable, where $I_i = 1$ if the individual was from the i th data source, otherwise $I_i = 0$; θ_1 , the fixed effect variable assigned to a demographic covariate; V_d , the apparent volume of distribution of efavirenz; \widehat{V}_d , the typical value of V_d ; μ , the mean value of the indicated demographic covariate; θ_2 , the fixed effect variable assigned to a genotypic covariate; θ_3 , the fixed effect variable assigned to a data source covariate (except for the one used in layer 2); OF, the minimum value of objective function; Δ OF, the change in OF of the model in each layer when compared to the OF of the chosen model (bolded) of the previous layer.

*Model is significant at $\alpha = 0.05$. **Model is significant at $\alpha = 0.01$.

Statistical significance was determined at $\alpha = 0.05$ (corresponding to Δ OF = -3.84). Because the results of proportional models were mostly very similar to those of the additive models, only the results of proportional models were shown. Demographic covariates were centered or normalized by the mean value before testing (see layer 1). The covariate *SEX* takes the value 0 for male subjects and 1 for female subjects.

model that became insignificant after including other covariates.

Furthermore, the parameter estimates and diagnostic plots were also examined during model development and on the final model. Visual predictive check and bootstrapping ($n = 10,000$) were performed using PsN to evaluate the final model.

Simulations

Simulations based on the final model and its parameter estimates of fixed and random effects were carried out using Microsoft Excel 2010 for Windows version 14.0.7116.5000 (Microsoft, Redmond, WA). Trials of 100 consecutive simulations were run for a population of mixed genotype (with frequency of the T allele set at 43%),¹⁰ as well as for each genotypic population. In each simulation, 1,000 individuals were simulated and the percentage of individuals with $C_{14h} < 1$ mg/L (P_1) and those with $C_{14h} < 4$ mg/L (P_4) were recorded. C_{14h} was chosen because the study by Marzolini et al.,³ which suggested the therapeutic target of 1–4 mg/L, developed the target by using

plasma samples collected at an average of 14 hours post-dose.³ The percentage of individuals with C_{14h} falling within 1–4 mg/L ($P_4 - P_1$) was then calculated. After 100 simulations were run, the mean, minimum, and maximum values of each of P_1 , P_4 , and $P_4 - P_1$ were obtained. Simulations of doses between 600 and 50 mg, with minimum decrements of 50 mg, were carried out to find the optimal dose.

Furthermore, PD simulations were performed based on a logistic regression model relating C_p to the probability of successful viral suppression and the probability of CNS toxicity developed by Siccardi et al.²⁰:

$$p = \frac{1}{1 + e^{-(A+B \log C_{8-16h})}}$$

where p is the probability of having the PD outcome (either viral suppression or CNS toxicity) and C_{8-16h} is the average C_p (in ng/mL) at 8–16 hours postdose. For the prediction of viral suppression, the PD parameters $A = -8.38$ (SE = 100%) and $B = 3.12$ (SE = 42%). As to CNS toxicity, $A = -6.65$ (SE = 43%) and $B = 1.68$ (SE = 51%).²⁰ The

Table 2 Demographic characteristics of the data according to the different sources

		Source no.				
		#1	#2	#3	#4	Combined
Sample size		79	9	61	14	163
Sex	Male (%)	69 (87.3)	5 (55.6)	56 (91.8)	13 (92.9)	143 (87.7)
	Female (%)	10 (12.7)	4 (44.4)	5 (8.2)	1 (7.1)	20 (12.3)
Age	Mean ± SD	46.0 ± 12.5	37.1 ± 6.6	41.6 ± 9.8	49.1 ± 9.9	44.1 ± 11.4
	Min/max	23/81	29/50	22/60	29/63	22/81
Body weight	Mean ± SD	64.7 ± 9.68	52.0 ± 6.65	62.9 ± 10.4	66.7 ± 11.8	63.5 ± 10.4
	Min/max	45.4/88.2	39.0/60.5	40.0/85.6	49.4/94.0	39.0/94.0
Body height	Mean ± SD	N/A	165.0 ± 3.94	167.8 ± 7.54	165.6 ± 7.30	167.0 ± 5.23
	Min/max	N/A	159/172	141/180	152/175	141/180
Nucleoside/nucleotide reverse transcriptase inhibitors	Lamivudine	38	N/A	53	11	102
	Zidovudine	20	N/A	1	4	25
	Stavudine	2	N/A	0	0	2
	Didanosine	4	N/A	0	0	4
	Abacavir	12	N/A	48	6	66
	Emtricitabine	35	N/A	7	3	45
	Tenofovir	44	N/A	11	3	58
Protease inhibitors	Lopinavir/ritonavir	2	N/A	0	1	3
CYP3A4 and CYP2B6 inducer	Rifampin	0	N/A	2	N/A	2
CYP3A4 inhibitor	Amlodipine	N/A	N/A	2	N/A	2
Other co-medications	Isoniazid	4	N/A	2	N/A	6
	Ethambutol	1	N/A	2	N/A	3
	Pyrazinamide	0	N/A	2	N/A	2
	Acyclovir	3	N/A	0	N/A	3
	Azithromycin	0	N/A	9	N/A	9
	Cotrimoxazole	4	N/A	15	N/A	19
	Pentamidine	2	N/A	0	N/A	2
	Metformin	N/A	N/A	2	N/A	2
	Gliclazide	N/A	N/A	2	N/A	2
	CYP2B6-G516T polymorphism	GG (%)	47 (59.5)	3 (33.3)	30 (49.2)	6 (42.9)
GT (%)		28 (35.4)	4 (44.4)	25 (41.0)	8 (57.1)	65 (39.9)
TT (%)		4 (5.1)	2 (22.2)	6 (9.8)	0 (0)	12 (7.3)

N/A, not available.

Source #1 contains data from routine therapeutic drug monitoring. Source #2 was from a clinical pharmacokinetic study of efavirenz. Source #3 was a study of the sleep quality of efavirenz-treated patients. Source #4 was a study of the comorbidity in human immunodeficiency virus (HIV)-infected patients. Co-medications with only one subject recorded are not shown.

treatment success rate was calculated by the following equation:

$$P_{\text{success}} = P_{\text{viral suppression}} \times (1 - P_{\text{CNS toxicity}})$$

Optimal doses determined from both methods (maximum $P_4 - P_1$ and the logistic regression model) were compared.

RESULTS

Excluded subjects

The study started with a total of 186 subjects before exclusions were made. Records without information about genotype or C_p were removed; all non-Chinese subjects were excluded; a further nine subjects were excluded as they had started on efavirenz for less than 7 days at the time of sampling. Two sampling records from two individuals were discounted because of suspected nonadherence (one being below the limit of quantification and the other was 0.4 mg/

L, which was much lower than other records from the same subject). The final dataset combining data from routine clinical service and three previous studies consisted of 163 individuals with a total of 266 records of C_p (see **Table 2** for the demographic details).

Demographic covariates analyses

Among sex, age, body weight and height, only body weight was found to be significantly associated with CL of efavirenz (change in the minimum value of objective function (ΔOF) = -5.632, $p=0.018$). No co-medications tested (see **Table 2**) was found to influence CL . No assignment of demographic covariates on volume of distribution (V_d) showed significance ($\Delta OF < 2.587$, $P > 0.11$ for all the covariates tested) (see **Table 1**, layer 1).

Genotypic covariate analyses

All parameterizations of the effects of CYP2B6-G516T polymorphisms on CL significantly improved the fit ($\Delta OF >$

Table 3 Final PK models and parameter estimates

The final model		Basic PK model			
		One-compartment model with first-order absorption			
		Interindividual variability			
		CL model: $CL = \widehat{CL}_0 \times [1 + \theta_{CYP2B6} \times \ln(g+1)] \times [1 + \theta_{WT} \times (WT/63.5 - 1)] \times (1 + \eta_{CL})$			
		V_d model: $V_d = \widehat{V}_d \times e^{\eta_v}$			
		$k_a = 0.445$ (fixed)			
		Intraindividual variability			
		$C_p = \widehat{C}_p \times (1 + I_4 \theta_{dataset\ 4}) \times (1 + \varepsilon)$			

Parameter	Estimate (RSE ^a)	Shrinkage	Bootstrap mean (90% CI ^d)	ΔOF in backward elimination	
CL model	\widehat{CL}_0 (L/h)	2.38 (7%)	/	2.39 (2.09–2.67)	/
	θ_{CYP2B6}	3.00 (10%)	/	3.00 (2.46–3.55)	+120.985
	θ_{WT}	0.461 (32%)	/	0.459 (0.209–0.712)	+8.593
	ω_{CL}	19.8% ^b (21% ^c)	34%	19.3% ^b (16.2%–22.8% ^e)	+25.239
V_d model	\widehat{V}_d (L)	221 (21%)	/	228 (125–317)	/
	ω_v	69.7% ^b (46% ^c)	75%	66.8% ^b (21.9%–96.2% ^e)	+9.161
Intraindividual variability	$\theta_{dataset\ #4}$ ^f	0.330 (40%)	/	–0.338 (–0.450 to –0.144)	+13.325
	σ	27.6% ^b (16% ^c)	15%	27.5% ^b (24.1%–30.6% ^e)	/

CI, confidence interval; CL, the apparent clearance of efavirenz; \widehat{CL}_0 , the typical value of CL among TT subjects; C_p , the steady state plasma levels of efavirenz; \widehat{C}_p , the predicted value of C_p ; g , the functional score of the CYP2B6-G516T polymorphism of the individual ($g = 0, 1$ and 2 for genotypes of TT, GT, and GG, respectively); k_a , the absorption rate constant of efavirenz; PK, pharmacokinetic; RSE, relative standard error; V_d , the apparent volume of distribution of efavirenz; \widehat{V}_d , the typical value of V_d ; θ , the fixed effect variable of the indicated covariate; η , the random effect variable of the indicated parameter (with mean zero and variance ω^2); I_4 , the source indicator variable, where $I_4 = 1$ if the individual was from data source #4, otherwise $I_4 = 0$; ε , the random effect variable of C_p (with mean zero and variance σ^2); ΔOF in backward elimination analyses, the change in the minimum value of objective function of the model when the covariate is removed from the final model.

^aRelative standard error of the estimate, which is obtained by dividing the standard error by the estimate; ^bEstimate of inter- and intraindividual variability expressed as coefficient of variation (CV) expressed as percentage; ^cSE of the CV expressed as percentage; ^d90% CI of the mean of estimates for bootstrap; ^e90% CI of the CV for bootstrap; ^fFixed effects were added to estimate the effect of biases in the data sources on intraindividual variability.

110.037, $P < 0.001$). The model using logarithmic function yielded the best fit ($\Delta OF = -119.56$, $P < 0.001$) (see **Table 1**, layer 2), in which typical CL of the GG, GT, and TT populations were estimated to be 10.6, 7.57, and 2.42 L/h, respectively. Assigning separate fixed effects to each genotype was not significantly better ($\Delta OF = -119.636$).

Biases between sources of data

A preliminary two-way analysis of variance revealed that both genotype and data source significantly contributed to the variation in C_p (see the **Supplementary Table S2**), and, therefore, we performed analyses on potential biases from data sources. No significant bias was identified in sources #1 and #3 ($\Delta OF < 2.963$, $P > 0.09$), whereas bias for CL or C_p from sources #2 and #4 were found to be significant ($\Delta OF = -4.421$, $P = 0.04$; $\Delta OF = -12.054$, $P < 0.001$, respectively) (see **Table 1**, layer 3). C_p in sources #2 and #4, were estimated to be 19.9% higher and 32.2% lower than the rest of the dataset.

Unexplained variability analyses

Further introduction of the additive error model to explain interindividual variability in CL did not improve the fit ($\Delta OF = 0.00$). Replacement of the proportional error model by the additive or the exponential error model worsened the fit. Introduction of error to explain interindividual variability in V_d revealed that exponential error model best described its distribution ($\Delta OF = -7.955$, $P = 0.005$, when

compared to a single estimate of V_d). Therefore, the exponential error model was assigned to V_d , whereas the proportional error model was used to describe variability in CL.

Applying a proportional-additive error model to explain intraindividual variability did not improve fitting ($\Delta OF = 0.00$) and replacing the proportional model by the additive error model worsened the fit. Therefore, the error model for residual variability remained to be the proportional error model.

The final model and parameter estimates

The richest model included body weight, CYP2B6-G516T genotype, and data source as covariates. During backward elimination, removing source #2 from the model revealed its insignificance ($\Delta OF = 4.186$, $P = 0.041$). Further removal of any remaining covariate showed that all of them remained significant (see **Table 3**).

In the final model, the typical CL of efavirenz for GG, GT, and TT carriers were estimated to be 10.2, 7.33, and 2.38 L/h, respectively. An increase (or decrease) of 1 kg in body weight from the mean value was estimated to increase (or decrease) CL of efavirenz by 0.73%. Unexplained biases between sources of data were also identified. The average C_p of source #4 was found to be significantly lower by 33.0% on average (see **Table 3**).

Final model evaluation

Shrinkages of random effects were investigated. The shrinkage of the random effect assigned to V_d was large

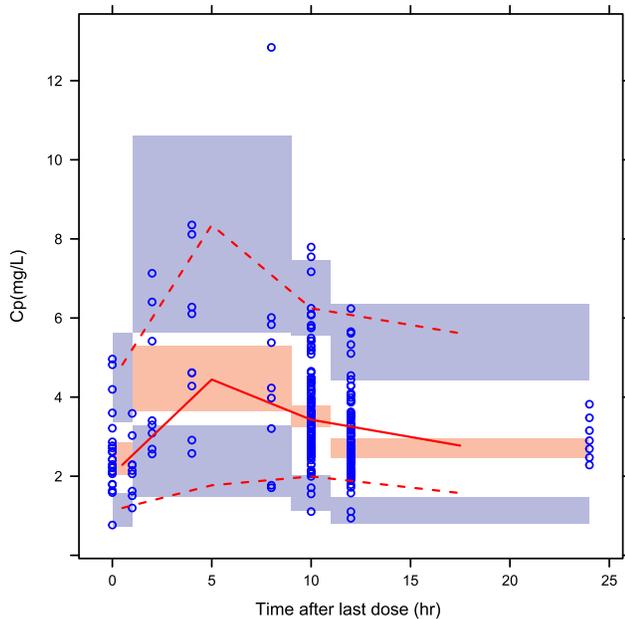


Figure 1 Prediction- and variance-corrected visual predictive check of the final model generated by PsN. The above plot compares the predicted plasma level profile with observed data, which are prediction- and variance-corrected for genotype and body weight. The blue shaded regions show the 90% confidence interval of the 5th and 95th percentiles of predicted plasma levels. The red shaded region shows the 90% confidence interval of the median of predicted plasma levels. The hollow circles show the prediction- and variance-corrected observations, and the red lines represent the best fit of corrected data.

(75%), which was expected because of sparse sampling (most subjects had only one sampling point between successive doses). The parameter estimates from bootstrapping were similar to those estimated with the original data (see **Table 3** for the shrinkages and results of bootstrap-

ping). Diagnostic plots showed satisfactory results (see the **Supplementary Figures S1.1** and **S1.2**). Prediction- and variance-corrected visual predictive checks (pvcVPC) were performed to correct for the differences coming from independent variables other than time. The pvcVPC suggested an accurately developed model (see **Figures 1** and **2**). Existence of an outlier at 8 hours postdose in the GT population in the pvcVPC could be attributed to the small number of data available at that time point.

Simulations

Simulation results showed that universal dosing of either 600 mg or 400 mg daily would result in lower rates of successful treatment than genotype-based dosing (mean percentages of individuals with C_p 14 hours postdose [C_{14h}] falling within 1–4 mg/L were 62.9% and 74.3%, respectively). Daily doses of 550 mg, 350 mg, and 100 mg would be optimal for GG, GT, and TT subjects, respectively (mean percentages of individuals with C_{14h} falling within 1–4 mg/L were 90.6%, 92.0%, and 91.8%, respectively), by targeting the 1–4 mg/L therapeutic range. When given daily oral doses of 400 mg, simulations revealed that the mean percentages of GG, GT, and TT subjects with C_{14h} falling within 1–4 mg/L were 85.5%, 91.8%, and 8.09%, respectively. These results showed that genotype-based dose individualization may be better than giving 400 mg for all¹¹ (see **Table 4** for the results of the above simulations and **Figure 3** for the typical plasma level profiles for each genotype at 600 mg daily, 400 mg daily, and at the optimal doses determined with respect to the 1–4 mg/L therapeutic range).

Rates of successful treatment were also simulated using the logistic regression model without variance proposed by Siccardi *et al.*²⁰ Dose optimizations showed that for the GG, GT, and TT populations, daily doses of 500 mg, 350 mg, and 100 mg would result in rates of successful treatment of 64.9%, 64.9%, and 64.7%, respectively (see

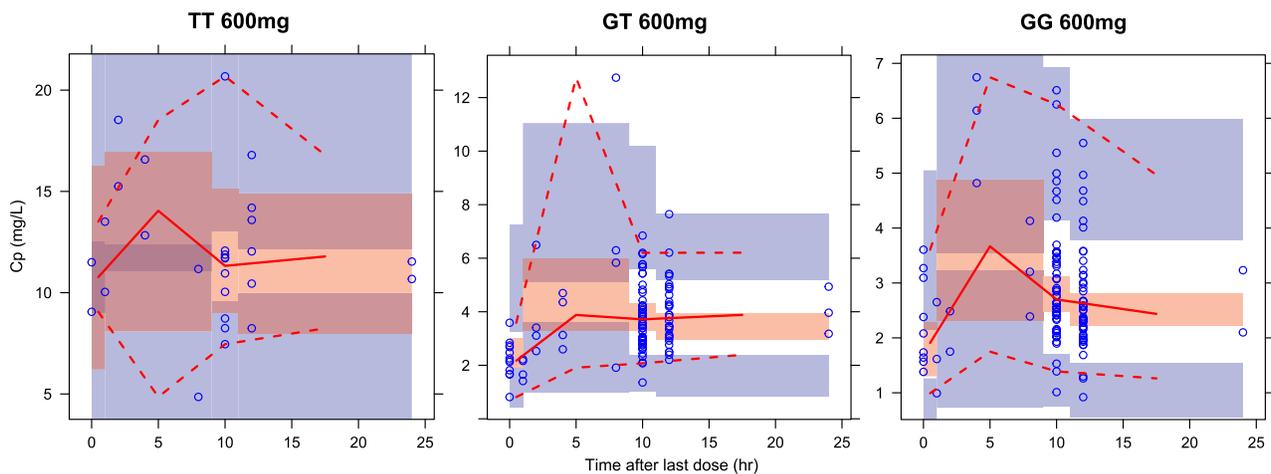


Figure 2 Prediction- and variance-corrected visual predictive check of the final model generated by PsN (with stratification by genotype). These plots are similar to the plot in **Figure 1**, except that plots for different genotypes are separated. The blue shaded regions show the 90% confidence interval of the 5th and 95th percentiles of predicted plasma levels. The red shaded region shows the 90% confidence interval of the median of predicted plasma levels. The hollow circles show the prediction- and variance-corrected observations, and the red lines represent the best fit of corrected data.

Table 4 Simulation results using EXCEL

Genotype	Daily dose (mg)	Results of simulations of rates of successful treatment using the therapeutic range of 1–4 mg/L			Results of simulations of rates of successful treatment using the logistic regression model
		P_1 mean (%) (min–max)	P_4 mean (%) (min–max)	$(P_4 - P_1)$ mean (%) (min–max)	Mean proportion of populations with treatment success (%)
All genotypes	600	1.44 (0.7–2.6)	64.4 (60.2–67.7)	62.9 (58.8–66.1)	59.9
	400	6.16 (4.3–8.2)	80.4 (76.8–83.4)	74.3 (70.9–77.8)	62.5
GG	600	4.14 (2.6–5.7)	93.7 (91.9–95.2)	89.5 (87.1–91.7)	64.3
	550	5.56 (3.8–7.4)	96.2 (94.7–97.6)	90.6 (88.4–93.1)	64.6
	500	7.50 (5.9–9.5)	97.9 (96.4–98.7)	90.4 (88.0–92.2)	64.8
	450	10.4 (7.6–13.0)	98.9 (97.9–99.6)	88.5 (85.9–91.6)	64.7
	400	14.0 (11.0–16.8)	99.5 (99.0–100)	85.5 (82.6–88.5)	64.6
GT	600	0.88 (0.2–1.7)	69.9 (66.2–74.2)	69.0 (65.7–73.2)	62.1
	400	3.57 (2.4–5.3)	95.3 (93.7–96.7)	91.8 (89.3–93.5)	64.6
	350	5.90 (4.5–7.5)	98.0 (96.8–99.1)	92.0 (89.8–94.0)	64.9
	300	10.6 (7.4–13.0)	99.3 (98.5–99.9)	88.7 (86.3–92.2)	64.7
TT	600	0 (0–0)	1.19 (0–2.7)	1.19 (0–2.7)	46.3
	400	0.10 (0–0.6)	8.19 (6.1–9.9)	8.09 (6.0–9.8)	53.1
	200	0.45 (0–0.9)	63.1 (57.5–66.3)	56.7 (65.9–62.7)	61.9
	150	1.32 (0.7–2.3)	88.5 (85.7–91.0)	87.2 (83.6–89.7)	64.1
	100	7.25 (5.6–9.1)	99.0 (97.9–99.9)	91.8 (89.9–93.3)	64.8
	50	63.3 (59.6–66.7)	99.99 (99.8–100)	33.3 (40.4–36.7)	58.1

Simulation results for populations with mixed genotypes (with frequency of the T allele set at 43%) are shown in the first two rows, followed by those with individual genotypes. Optimal doses for different genotypes determined from each method are bolded.

P_1 , the percentage of individuals with steady state plasma level of efavirenz 14 hours postdose <1 mg/L; P_4 , the percentage of individuals with steady state plasma level of efavirenz 14 hours postdose <4 mg/L; $P_4 - P_1$, the percentage of individuals with steady state plasma level of efavirenz 14 hours postdose falling within 1–4 mg/L.

Table 4). We also simulated variabilities in PD parameters using the reported SEs, but the results were uninformative because the simulated probabilities varied greatly between simulations and were indistinguishable between doses. For instance, three simulations using each of 50 and 1,000 mg of efavirenz orally daily in the GG population resulted in ranges of percentages of individuals with successful treatment of 33.6–39.0% and 34.0–42.2%, respectively. This was very likely because of the unreasonably high SEs in the PD parameter estimates reported in Siccardi *et al.*²⁰

DISCUSSION

This study is the first one attempting to assess the effects of multiple factors, including CYP2B6-G516T polymorphisms and demographic factors, on the PKs of efavirenz in the Chinese population. The results generated allowed us to quantify the effects of different covariates on C_p , as well as to predict accordingly the doses for patients with different CYP2B6-G516T genotypes that are likely to improve patient outcomes by ensuring sufficient viral suppression while minimizing CNS toxicity.

Limitations in parameter estimations

Of the 163 individuals in the database, only 52 presented with multiple C_p records and only 9 presented with inten-

sive multiple samplings. Therefore, there was a lack of absorption-phase data and overparameterization was encountered during model development when k_a , CL , and V_d were simultaneously estimated. In light of this, we used a more parsimonious minimum model by pre-estimating instead of estimating k_a during model development. Despite this, the k_a value we used was consistent with reported values in literature (see **Supplementary Table S3**). The relatively large estimate of intraindividual variability can also be explained by the fact that some C_p records did not have the exact time of sampling, and they were replaced by the estimated time of sampling.

In addition, despite that efavirenz concentrations are known to be influenced by other factors, such as other CYP2B6 polymorphisms and CYP2A6/3A4 and ABCB1 polymorphisms, as reported previously,^{18,21} relevant genotypic data were not available such that their effects could not be assessed in this study. Furthermore, as illustrated in the studies by Pfister *et al.*²² and by Kappelhoff *et al.*,²³ respectively, a lag time introduced into the model as a covariate might improve the fit. However, because of the fact that some subjects did not present with complete records regarding the timings of samplings, adding time lag as a covariate is unlikely to be meaningful.

The final PK model and parameter estimates

The final PK model and parameter estimates obtained were compared to those reported in other studies available in

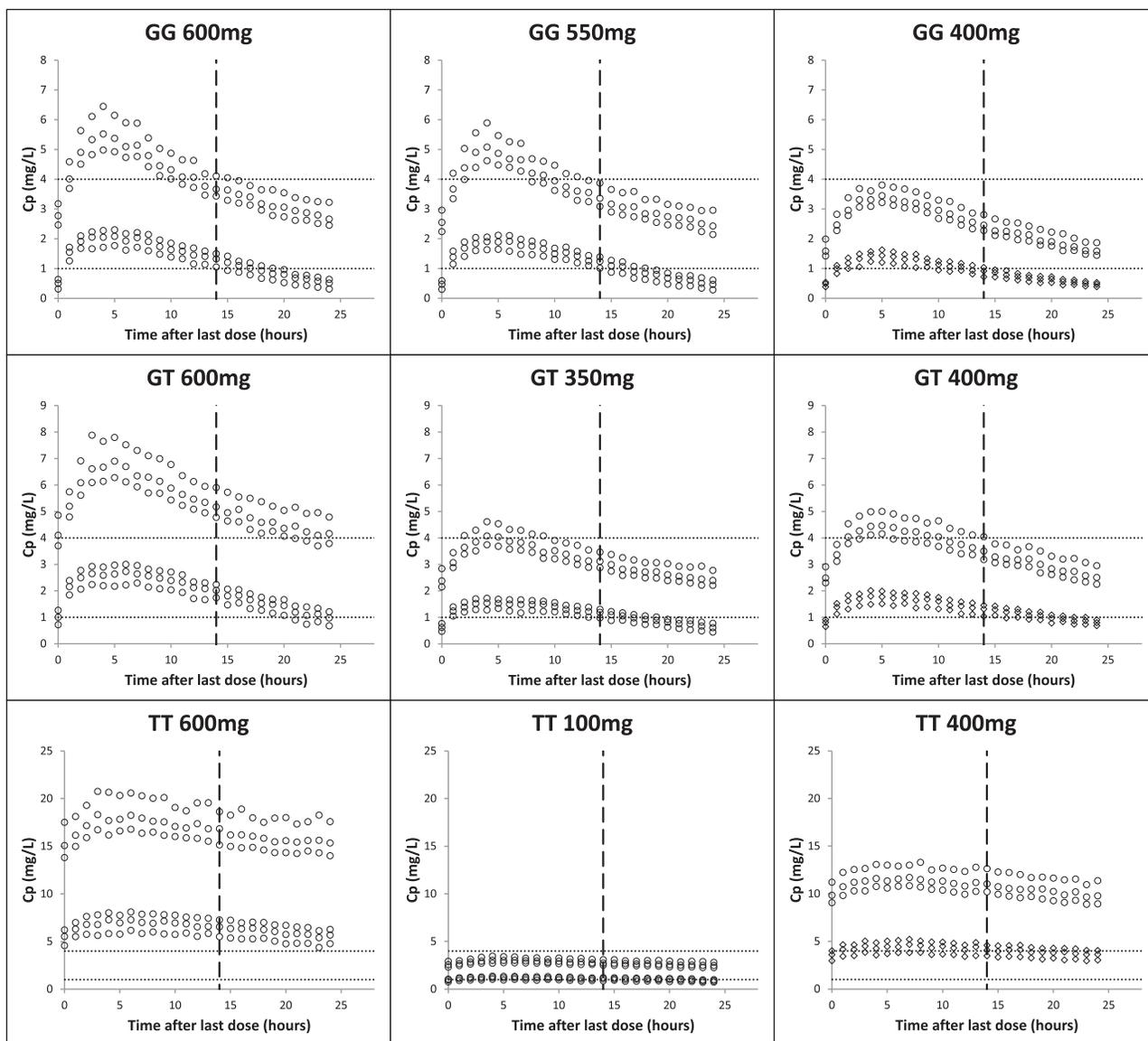


Figure 3 C_p profiles for each genotype at 600 mg daily, the optimal dose, and 400 mg daily. The horizontal dotted lines show the range of C_p of 1–4 mg/L. The vertical dotted line marks the time of 14 hours after the last dose. The hollow circles show the 95th, 90th, 85th, 15th, 10th, and 5th percentiles of C_p , respectively.

current literature and the results were found to be similar (see the **Supplementary Table S3**).^{18–20,24} As reported in several studies, this study showed that body weight has a significant effect on CL of efavirenz,^{18,22,25–27} whereas some studies reported negative findings.^{19,24} Previous findings on the effect of sex on CL of efavirenz^{24,25,28} could not be confirmed in this study and a few other studies.^{19,22} In fact, the failure to confirm the association can be attributed to the small proportion of female subjects ($n=20$; 12.3%) in our combined database. This possibility is further substantiated by the fact that two studies carried out by Pfister *et al.*²² and Kappelhoff *et al.*,²³ respectively, with few female subjects ($n=12$; 8.6% and $n=25$; 16.7%, respectively) could not confirm the effect, whereas two other studies car-

ried out by Burger *et al.*²⁸ and Nyakutira *et al.*,²⁴ respectively, with more female subjects ($n=66$; 25.9% and $n=45$; 63.4%, respectively) managed to show that the effect was significant. The findings from this study that age and body height have no significant effect on CL of efavirenz are consistent with previous findings.^{19,22,23} No co-medications were found to significantly influence C_p . Although rifampin is well known to be a strong CYP2B6 inducer, its effect on C_p could not be confirmed in this study, probably because of the fact that only two subjects were recorded to be taking the drug. The strong association between CYP2B6-G516T polymorphisms and CL of efavirenz shown in this study was very much expected and consistent with previous findings.^{13,18,24}

Biases between sources of data

The bias from source #4 remains unexplained by the covariates tested. We note that subjects in source #4 have a higher proportion of long-term patients with significant comorbidities, such as hyperlipidemia. It is also suspected that more complete enzyme autoinduction (for both CYP2B6 and potentially also CYP3A4), or co-medications, such as statins, might explain the apparently lower C_p . However, these suppositions could not be confirmed because of the lack of complete patient records. Meanwhile, any bias in the timing of sampling in source #4 might have contributed to such systemic bias in C_p as well. Although combining multiple sources is a limitation of the current analyses, the inclusion of the data source as a covariate enabled us to estimate the effect of genotype on CL independent of the sources of the subjects. Hence, our estimated effect of the genotype is more robust across the different study populations.

Simulations

Considering the determination of optimal doses by targeting the 1–4 mg/L therapeutic range, it could be observed that for each genotype a range of doses actually produced similar outcomes (see **Table 4**). Furthermore, the optimal doses to target the 1–4 mg/L therapeutic range were generally similar to those generated by using the logistic regression PD model. These showed the agreement between the 1–4 mg/L therapeutic range and the logistic regression PD model in predicting PD outcomes given the C_p profiles simulated by our developed model.

Clinical relevance

Despite the fact that CYP2B6-G516T polymorphism is a major predictor of the optimal doses, the recommended standard dose for efavirenz is 600 mg daily for all patients, regardless of CYP2B6 genotype. Although the ENCORE1 study suggested the use of a reduced dose universally is noninferior to the standard dose,¹¹ the current study provides evidence that genotype-based dose individualization can potentially lead to a higher probability of successful viral suppression without unwanted CNS effects, which may translate to improved clinical outcomes. Considering the fact that efavirenz is only available in 200 mg and 600 mg tablets, GG patients can continue their 600 mg daily treatment, whereas GT patients can be prescribed 400 mg daily. TT patients can be started on 200 mg daily, and if CNS effects are troubling and therapeutic drug monitoring reveals C_p above 4 mg/L, the dose can be further reduced to 100 mg. However, the lack of co-formulation of other nucleoside reverse transcriptase inhibitors with reduced strength efavirenz represents a significant inconvenience to the patient and, therefore, the implementation of the reduced doses of efavirenz remains challenging.

CONCLUSION

The results of the study provided a PK model to allow predictions of C_p and PD outcomes according to patients' demographic and genotypic details. It showed that pharmacogenetics-based dose individualization of efavirenz

can be leveraged to help optimize patient outcomes. The present study serves as important scientific evidence supporting the clinical application of efavirenz dose individualization based on the CYP2B6 genotype; further clinical study on the clinical outcomes of dose individualization of efavirenz is being conducted to verify our findings.

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