


Pharmacokinetics and Drug-Drug Interactions of Isoniazid and Efavirenz in Pregnant Women Living With HIV in High TB Incidence Settings: Importance of Genotyping

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The World Health Organization guidelines recommend that individuals living with HIV receive ≥ 6 months of isoniazid preventive therapy, including pregnant women. Yet, plasma isoniazid exposure during pregnancy, in the antiretroviral therapy era, has not been well-described. We investigated pregnancy-induced and pharmacogenetic-associated pharmacokinetic changes and drug-drug interactions between isoniazid and efavirenz in pregnant women. Eight hundred forty-seven women received isoniazid for 28 weeks, either during pregnancy or at 12 weeks postpartum, and 786 women received efavirenz. After adjusting for *NAT2* and *CYP2B6* genotype and weight, pregnancy increased isoniazid and efavirenz clearance by 26% and 15%, respectively. Isoniazid decreased efavirenz clearance by 7% in *CYP2B6* normal metabolizers and 13% in slow and intermediate metabolizers. Overall, both isoniazid and efavirenz exposures were reduced during pregnancy, but the main determinants of drug concentration were *NAT2* and *CYP2B6* genotypes, which resulted in a five-fold difference for both drugs between rapid and slow metabolizers.

Study Highlight

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Genetic variability greatly impacts isoniazid and efavirenz exposure, and it may affect interactions between these two commonly co-administered drugs. Very little knowledge is available on the effect of pregnancy on their pharmacokinetics (PKs).

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ We investigated the magnitude of pregnancy-induced changes in isoniazid and efavirenz PKs, accounting for drug-drug interactions and pharmacogenetics.

WHAT DOES THIS STUDY ADDS TO OUR KNOWLEDGE?

☑ Pregnancy increases clearance of isoniazid (by 26%) and efavirenz (by 15%), reducing both drug's exposures.

Isoniazid co-administration reduces efavirenz clearance, and this reduction is larger in slow and intermediate *CYP2B6* metabolizer.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The modest pregnancy-related decreases in both drugs exposures may be of clinical importance in rapid *NAT2* acetylator or *CYP2B6* metabolizer, who already experience low exposure and are, hence, at risk of treatment failure. Isoniazid-induced increase in efavirenz exposure may raise the chance of toxicity among slow *CYP2B6* metabolizers. Both findings suggest potential role for genetic testing and therapeutic drug monitoring.

HIV and tuberculosis are leading causes of morbidity and mortality among women of reproductive age, particularly among those living in low-income and middle-income countries.^{1,2} Pregnant women living with HIV have an increased susceptibility to tuberculosis (TB) infection and progression from latent

TB infection to active disease.³ To reduce the risk of HIV-associated TB, the World Health Organization (WHO) recommends 6 months of isoniazid preventive treatment (IPT) along with antiretroviral therapy (ART).⁴ This recommendation was also made for pregnant women, based on limited data. Both

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antiretrovirals and anti-TB regimens often include drugs that inhibit or induce liver enzymes, including cytochrome-P450 (CYP) isoenzymes, causing potential drug-drug interactions (DDIs).⁵ The situation is further complicated in pregnancy, which is associated with physiological changes, altered body weight and composition, changes in plasma protein concentrations, and other processes that can affect drug pharmacokinetics (PKs).^{6–9}

The metabolism of both efavirenz (a commonly used antiretroviral to treat HIV) and isoniazid are affected by genetic polymorphisms in specific drug-metabolizing genes, and plasma drug levels can be further influenced by pregnancy. Studies to date, although small in number of participants and limited in geographic diversity have shown, a predicted two-fold increase in *CYP2B6* activity during pregnancy.⁷ Dooley *et al.*¹⁰ observed a 19% increase in plasma efavirenz clearance during pregnancy compared with the postpartum period, and Olagunju *et al.*¹¹ reported similar findings. Furthermore, an ancillary metabolic pathway of efavirenz, *CYP2A6*, is inhibited by isoniazid, thus potentially causing DDIs.¹² There is currently little data regarding the effect of pharmacogenomics and pregnancy on isoniazid PKs and on the DDI between isoniazid and efavirenz in pregnancy and postpartum.

As part of the recently completely IMPAACT study, P1078 TB APPRISE trial, which investigated the safety and efficacy of administering IPT to pregnant women living with HIV, and reported a significantly higher rate of adverse pregnancy outcomes in women who received IPT during pregnancy,¹³ we analyzed the key pharmacogenetic polymorphisms of efavirenz and isoniazid metabolism, the changes in the drug concentrations and PKs of these two drugs during pregnancy, and their DDIs.

METHODS

Participants and study design

This analysis used PK data collected in IMPAACT study P1078. The study design is reported in the primary manuscript¹³ summarized here, and we report the PK procedure. The IMPAACT study P1078 was a prospective, double-blind, placebo-controlled, randomized, noninferiority trial conducted in 8 countries, and had a total of 13 sites. Local and collaborating institutional review boards approved the trial, and all women

provided informed consent. Full details on ethics approval are provided in the primary manuscript.¹³

HIV-positive pregnant women ≥ 18 years old were recruited if gestational age was 14 to 34 weeks, on WHO-recommended HIV treatment for prevention of mother-to-child transmission, weighing > 35 kg. At study entry, women were randomized either to arm A (immediate 28 weeks of 300-mg isoniazid daily treatment, then placebo) or arm B (deferred isoniazid treatment, placebo until week 12 postpartum, then 28 weeks of 300 mg isoniazid). Adherence to isoniazid (or placebo) was monitored by self-report and pill count defined as the percentage of expected doses/pills taken during the entire treatment period, whereas ART adherence was self-reported.

Sample collection

Intensive samples were captured at predose, 1, 2, 4, 6, 8, and 12 hours postdosing, whereas sparse sampling occurred at least 2 hours postdosing. Both sparse and intensive samples were captured on two visits; during pregnancy (between 28 and 40 weeks' gestation) and week 16 postpartum (+ 4 weeks). Blood samples were centrifuged at 800 G for 10 minutes, and plasma was aliquoted into cryovials and immediately frozen at -70°C to await further processing. Plasma concentrations were determined by liquid chromatography-tandem mass spectrometry. Isoniazid calibration ranged from 0.105 to 25.0 mg/L, the interday accuracy and precision ranged from 92.2% to 104.5%, and 6.5% to 10.8%, respectively. Efavirenz calibration ranged from 0.0195 to 20.0 mg/L, the interday accuracy and precision ranged from 95.2% to 100.2%, and 3.2% to 11.3%, respectively.

Composite *CYP2B6* genotype was defined based on combinations of four polymorphisms as follows: normal (1: 15582CC-516GG-983TT or 2: 15582CT-516GG-983TT); intermediate (3: 15582TT-516GG-983TT; 4: 15582CC-516GT-983TT; 5: 15582CC-516GG-983CT; 6: 15582CT-516GT-983TT; or 7: 15582CT-516GG-983CT); and slow metabolizer genotype (8: 15582CC-516TT-983TT; 9: 15582CC-516GT-983CT; 10: 15582CC-516GG-983CC, each with or without 11: -48GT ; and 12: -48GG).¹⁴ Individuals with 983CC were considered ultra-slow metabolizers. (The term “normal” is used for consistency with standard nomenclature, not to suggest that others are abnormal.) For *NAT2*, genotypes were categorized based on combinations of rs1801279 (*NAT2*14*), rs1801280 (*NAT2*5*), rs1799930 (*NAT2*6*), and rs1799931 (*NAT2*7*), as slow, homozygous for the variant allele at any of the four loci (i.e., AA, CC, AA, and AA, respectively), or heterozygous at two or more loci; intermediate, heterozygous at a single locus; or rapid, no variant allele at any locus (i.e., GG, TT, GG, and GG, respectively).^{15,16} Genotyping was done in Vanderbilt Technology for Advanced Genomics (VANTAGE) using MassARRAY iPLEX

Gold (Agena Bioscience, San Diego, CA) and Taqman (ThermoFisher Scientific, Waltham, MA).

Data analysis

Isoniazid and efavirenz concentrations were interpreted using population PK modeling using NONMEM version 7.4.3.¹⁷ Perl-speaks-NONMEM version 4.8.1, Pirana, and R with the package Xpose4 were used to facilitate the model development process, data manipulation, and generation of model diagnostics.¹⁸ To describe the PK of both isoniazid and efavirenz, one-compartment and two-compartment models were tested with first-order absorption (with or without lag time or a chain of transit compartments), and first-order elimination. Because both drugs are mainly hepatically cleared, a well-stirred liver model¹⁹ was tested to capture the effect of first-pass metabolism. The liver hepatic blood flow Q_h ²⁰ was assumed to be 90 L/h in a typical individual and adjusted for the effect of body size using allometric scaling.²¹ The free fraction of efavirenz and isoniazid in plasma were fixed to 0.5%²² and 95%,²³ respectively. The prehepatic bioavailability of a typical individual was fixed to a reference value of 1.

The intensive data was used to develop the base model because these women were monitored closely, dosing was observed, and richer sampling schedule allowed for the identification of the structural model. This model was then used to explore the sparse data and identify implausible values, which were flagged as outliers and removed from model development analysis. An extreme value of conditional weighted residuals²⁴ (i.e., absolute value ≥ 4) was used as a criterion to identify outliers. Because conditional weighted residuals values for data observations arising from the postulated model are assumed to be normally distributed, randomly obtaining an absolute value ≥ 4 has a probability of $< 0.00633\%$, only a chance of 1 of 10,000. We assumed that PKs of both drugs were at steady-state at the time of the PK visit unless the participant had been on efavirenz for < 16 days,²⁵ or missed dose in the previous 3 days had been reported.

Random effects at occasion-level (each dose), visit-level (each PK sampling visit), and/or subject-level were included on the PK parameters if statistically significant using a lognormal distribution. Between-occasion variability was tested on absorption parameters, whereas between-visit variability and between-subject variability (BSV) were tested on disposition parameters. A combination of proportional and additive error was used to model unexplained residual variability.

Model development was guided by evaluating the drops in objective function value (Δ OFV) of nested models. The OFV was assumed as χ^2 -distributed, hence with a 3.84 drop in OFV being significant at $P < 0.05$ for one additional parameter (i.e., 1 degree of freedom). Besides statistical significance, a set of diagnostic plots, including visual prediction checks and physiological plausibility of the results were considered in model development decisions.

Because enzyme metabolic status has been widely reported to critically affect PKs of both drugs, *NAT2* genotype effect on isoniazid clearance and *CYP2B6* and *CYP2A6* genotype on efavirenz clearance were tested early in the model development process.^{12,26} Allometric scaling was applied on all clearance and volume of distribution parameters²⁷ to account for body size effect. Other covariate effects on the PK parameters, including pregnancy and DDI, were investigated using a stepwise approach with forward inclusion ($P < 0.05$) and backward elimination ($P < 0.01$).

Concentrations below limit of quantification were handled similarly to the M6 method by Beal,²⁸ to mitigate the effect of the M6 method imputation the additive error for the imputed values was inflated by lower limit of quantification/2. Only a subset of participants was included in the genotype study, those with missing genotypic information were assigned a phenotype using the mixture model.²⁹ Participants with missing weight and height were assigned the typical values depending on whether pregnant or postpartum. The original protocol focused on IPT, hence did not include capturing ART PK information (i.e., efavirenz

dosing time) for sparse sampling. This was later amended, resulting in 32% of the women having a missing efavirenz dosing time in at least one PK visit during the early phase of the trial. Women with missing dose times during one visit were assumed to take medication at the same time throughout the trial consistently; therefore, dosing time of the recorded visit was imputed on the missing visit. For women with missing dosing time at both visits, a median dosing time (i.e., 20:30) from the data was imputed.

RESULTS

Study profile

PK samples were available from 847 women on 300 mg isoniazid (748 of whom were treated with efavirenz (600 mg dose), 80 with nevirapine, 17 with lopinavir, and 2 with atazanavir based ART. Of the 847 on isoniazid, 32 underwent intensive sampling (at predose, 1, 2, 4, 6, 8, and 12 hours postdosing), providing 300 observations, whereas 815 underwent sparse sampling (at least 2 hours postdosing), providing 1,015 observations, of which 88 were identified as implausible outliers (outliers were identified per the description in Methods) and were removed from the model-building process. Two hundred ten women had isoniazid profiles available during both pregnancy and postpartum, because they had started isoniazid at a late gestational age such that their 28 weeks of isoniazid had not elapsed prior to their postpartum PK visit.

The number of participants on efavirenz in the different arms (isoniazid or placebo treatment) varied because of ART switching over the course of the study, but the majority were on efavirenz. Of 786 women on efavirenz, 21 underwent intensive sampling, providing 266 observations, and 765 underwent sparse sampling, providing 1,363 observations, of which 15 were identified as implausible outliers (outliers were identified per the description in Methods) and were removed from the model-building process. Baseline characteristics of all women are summarized in **Table 1**.

Distribution of drug metabolizer genotypes

The distribution of drug metabolizer genotypes for *NAT2*, *CYP2B6*, and *CYP2A6* (classified into three phenotypes as described in Methods), stratified by country, is illustrated in **Figure 1**. Rapid isoniazid acetylator *NAT2* genotypes (had site range of 4–31%) were the least prevalent group in all eight countries, which varied considerably by sites. Intermediate metabolizer *CYP2B6* (had site range of 43–63%), were frequent in all countries genotypes as were *CYP2A6* normal metabolizer genotypes (had site range of 76–93%).

Structural model

A two-compartment disposition model best described the PKs of both isoniazid and efavirenz with first-order absorption through a chain of transit compartments.³⁰ Drug elimination implemented with a well-stirred liver model,¹⁹ was able to describe both hepatic clearance and first-pass extraction with the parameter of hepatic intrinsic clearance, as shown in **Figure 2**. Parameter estimates for isoniazid and efavirenz, and the precisions for each are presented in **Table 2** and **Table 3**, respectively.

Table 1 Demographic, clinical, and laboratory characteristics of women on isoniazid and efavirenz during pregnancy and at postpartum

Characteristics (median and range, or <i>n</i> and %)	Participants in isoniazid PK analysis		Participants in efavirenz PK analysis	
	Pregnancy (<i>n</i> = 420)	Postpartum (<i>n</i> = 637)	Pregnancy (<i>n</i> = 712)	Postpartum (<i>n</i> = 670)
Age, years	29 (18–45)	29 (19–42)	29 (18–45)	29 (18–45)
Weight, kg	68 (39–167)	62 (38–165)	67 (42–164)	61 (37–114)
Body mass index, kg/m ²	27 (18–61)	24 (16–45)	27 (18–61)	25 (16–49)
Gestation/postnatal age, weeks, at PK sampling time	26 (14–34)	16 (7–23)	26 (14–34)	16 (7–23)
Baseline viral load, copies/mL	< 40 (< 40–237,000)	< 40 (< 40–465,000)	< 40 (< 40–237,000)	< 40 (< 40–465,000)
Drug regimen				
On isoniazid	420 (100%)	637 (100%)	352 (49%)	540 (80%)
On efavirenz	371 (88%)	563 (88%)	712 (100%)	670 (100%)
On nevirapine	11 (3%)	47 (8%)	-	-
On lopinavir/ritonavir	5 (1%)	5 (1%)	-	-
On atazanavir/ritonavir	2 (0%)	2 (0%)	-	-
Days on EFV at PK sampling time	125 (18–3,800)	264 (1–4,228)	125 (18–3,800)	408 (1–4,228)
Drug metabolizing genotype				
	NAT 2 acetylation status		CYP2B6 metabolizer status	
Rapid	52 (12%)	70 (11%)	168 (24%)	146 (22%)
Intermediate	140 (33%)	202 (32%)	299 (42%)	264 (40%)
Slow	159 (39%)	199 (31%)	118 (16%)	102 (15%)
Ultra-slow	-	-	1 (0%)	2 (0%)
Missing	69 (16%)	166 (26%)	126 (18%)	156 (23%)
			CYP2A6 metabolizer status	
Normal			501 (70%)	439 (65%)
Intermediate			81 (11%)	71 (11%)
Slow			4 (1%)	4 (1%)
Missing			126 (18%)	156 (23%)

Two hundred ten women had isoniazid profiles during both pregnancy and postpartum.

Five hundred ninety-six women had efavirenz profiles available during both pregnancy and postpartum.

EFV, efavirenz; PK, pharmacokinetic.

Isoniazid pharmacokinetics: Covariate effects

Allometric scaling was applied to all clearance, volume, and liver parameters to account for body size effect, improving the fit, and explaining part of BSV ($\Delta\text{OFV} = -44$). When fat-free mass replaced bodyweight for allometric scaling of clearance (but not volume) parameters, it improved the fit of the model ($\Delta\text{OFV} = -6.27$). *NAT2* genotype significantly affected clearance of isoniazid ($\Delta\text{OFV} = -273$, χ^2 df = 2, $P \ll 0.001$), as intrinsic clearance varied greatly among rapid, intermediate, and slow acetylators. After adjusting for body size and *NAT2* genotype, pregnancy was found to increase clearance by 26% ($\Delta\text{OFV} = -49.6$, χ^2 df = 1, $P \ll 0.001$). There was no significant difference in clearance among participants on the four different ART (efavirenz, nevirapine-based, lopinavir/ritonavir-based, and atazanavir/ritonavir-based) regimens ($\Delta\text{OFV} = -6.34$, χ^2 df = 3, $P = 0.0964$). Inclusion of pregnancy on any other parameter, including the central compartment volume of distribution did not improve model fit.

Figure 3 displays the model-predicted individual exposures of isoniazid stratified by both *NAT2* genotype and pregnancy

status. The 0–24-hour area under the concentration-time curve (AUC_{0-24}) of isoniazid boxplot shows that isoniazid exposure differs among the three genotypes, and decreased exposures were observed during antepartum compared with postpartum in all three genotypes. Combining all genotypes, the overall median (interquartile range) of isoniazid AUC_{0-24} antepartum was 8.05 mg-h/L (4.43–16.7 mg-h/L), compared with 11.1 mg-h/L (6.26–23.9 mg-h/L) postpartum. Maximum concentrations (C_{max}) during pregnancy and postpartum were 2.89 mg/L (1.97–4.13 mg/L) and 3.69 mg/L (2.64–5.13 mg/L), respectively. The postpartum AUC_{0-24} was 1.4-fold greater compared with antepartum, whereas slow acetylator AUC_{0-24} was 5-fold greater compared with rapid acetylators. An isoniazid AUC_{0-24} of 10.52 mg-h/L has been associated with 90% of early bactericidal activity in patients with active TB.³¹

Efavirenz pharmacokinetics: Covariate effects

Efavirenz had a structural model similar to isoniazid, except the first-order absorption rate constant was unstable when sparse

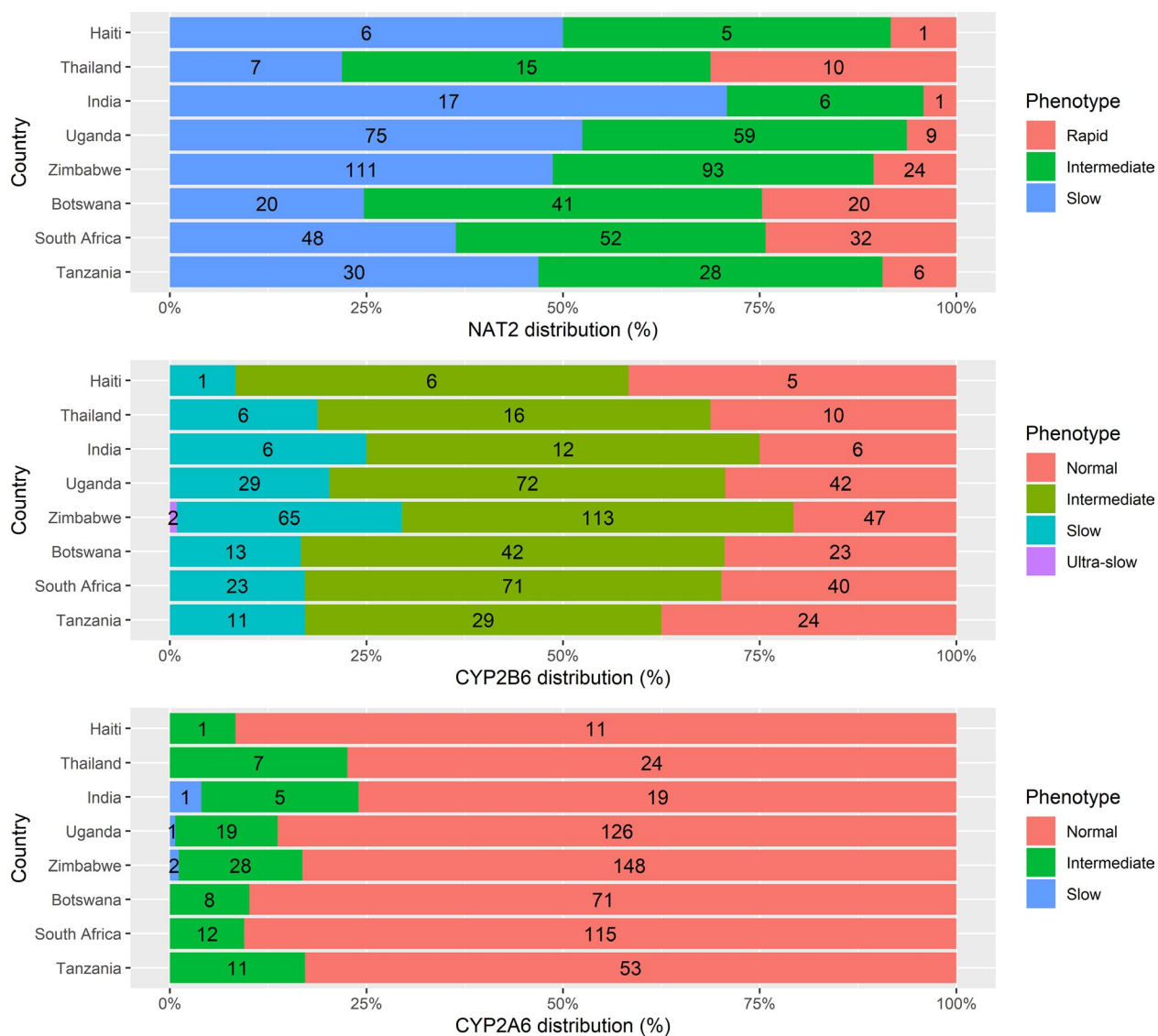


Figure 1 Distribution of the enzyme metabolizer genotypes for NAT2, CYP2B6, and CYP2A6 in the participants across the eight countries involved in the study. [Colour figure can be viewed at wileyonlinelibrary.com]

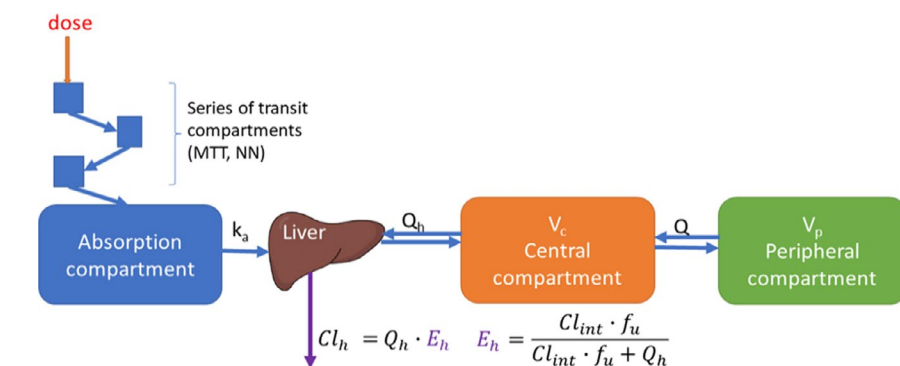


Figure 2 Schematic representation of the pharmacokinetic model of both efavirenz and isoniazid. The absorption is described with a series of transit-compartment to capture the delay in absorption, and a rate constant k_a . The hepatic extraction (E_h) is responsible for both first-pass metabolism and the systemic elimination with first-order kinetics. V_c represents the volume of distribution in the central compartment. Drug transfer between the central and peripheral compartment is defined by intercompartmental clearance Q/F , were F represents the oral bioavailability. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 2 Final PK parameter estimates for isoniazid

Parameter	Typical value (95% CI ^a)	Variability ^b , %CV (95% CI ^a)
CL _{int} ^c , L/hour, NAT2 rapid	72.3 (61.5–86.7)	69.2 (64.2–74.2) ^e
CL _{int} ^c , L/hour, NAT2 intermediate	38.5 (34.6–43.2)	
CL _{int} ^c , L/hour, NAT2 slow	14.5 (13.1–16.0)	
V _c ^d , L	37.6 (33.9–40.7)	
V _p ^d , L	13.3 (10.5–16.9)	
Q/F ^c , L/hour	3.32 (2.53–4.54)	
k _a , 1/hour	2.69 (1.91–3.51)	145 (116–172) ^f
MTT, hours	0.342 (0.209–0.459)	116 (98.7–150) ^f
NN	48.4 (22.2–83.8)	
Q _H ^c , L/hour	90 FIXED	
f _u , %	95 FIXED	
Prehepatic relative bioavailability	1 FIXED	12.3 (8.20–15.7) ^f
Proportional error, %	13.2 (11.3–15.3)	
Additive error, mg/L	0.0378 (0.0335–0.0449)	
Pregnancy effect on CL, %	+26.2 (19.8–33.2)	

%CV, percent coefficient of variation; CI, confidence interval; CL, clearance; CL_{int}, clearance intrinsic; f_u, unbound fraction of isoniazid in plasma 50; INH, isoniazid; k_a, first-order rate constant of INH absorption; MTT, absorption mean transit time; NN, number of absorption transit compartment; PK, pharmacokinetic; Q/F, apparent intercompartmental clearance for INH; Q_H, blood liver flow 40; V_c, apparent central volume of distribution for INH; V_p, apparent peripheral volume of distribution for INH.

The bold values represent significant covariates in the models.

^aThe 95% CIs were obtained with the Standardized Infection Ratio procedure.

^bVariability was modeled with log-normal distribution and is presented as an approximate percentage CV. ^cClearance parameters are allometrically scaled based on fat-free mass (typical value reported for 39 kg, which was the median fat-free mass weight of the study population). ^dVolume of distribution parameters are scaled based on weight (typical value reported for 67 kg, which was the median weight of the study population). ^eBetween subject variability. ^fBetween occasion variability.

and intensive data were pooled together; so, the value observed in the intensive PK data analysis was used as a fixed constant. Allometric scaling with bodyweight on all clearance and volume parameters improved the model fit and explained part of the BSV ($\Delta\text{OFV} = -28.6$). Using fat-free mass instead of total bodyweight for clearance (but not volume) parameters further improved the model ($\Delta\text{OFV} = -12.5$). *CYP2B6* genotype significantly affected the clearance of efavirenz ($\Delta\text{OFV} = -399$, χ^2 df = 2, $P \ll 0.001$). After adjusting for body size and *CYP2B6* genotype, pregnancy was found to increase the clearance of efavirenz by 16% ($\Delta\text{OFV} = -75.5$, χ^2 df = 1, $P \ll 0.001$). Clearance in women co-administrated isoniazid and efavirenz was 7% lower in normal metabolizers and 13% lower in slow and intermediate metabolizers ($\Delta\text{OFV} = -39.2$, χ^2 df = 2, $P \ll 0.001$) regardless of pregnancy status. Effect of *CYP2A6* phenotype on the clearance and bioavailability of efavirenz was investigated among *CYP2B6* slow metabolizers, in whom *CYP2A6* may be

Table 3 Final parameter estimates for efavirenz

Parameter	Typical value (95% CI ^a)	Variability ^b , %CV (95% CI)
CL _{int} ^c , L/hour, <i>CYP2B6</i> normal	2,690 (2,300–3,030)	53.8 (48.9–59.2) ^e
CL _{int} ^c , L/hour, <i>CYP2B6</i> intermediate	1,940 (1,790–2,100)	
CL _{int} ^c , L/hour, <i>CYP2B6</i> slow	545 (487–624)	
V _c ^d , L	135 (109–165)	
V _p ^d , L	512 (487–623)	
Q/F ^c , L/hour	26.9 (19.8–36.5)	
K _a , 1/hour	1.75 Fixed	180 (114.9–227) ^f
MTT, hour	1.78 (1.20–2.39)	131 (103–166) ^f
NN	48.4 (11.3–64.7)	
Q _H ^c , L/hour	90 Fixed	
f _u , %	0.5 Fixed	
Prehepatic relative bioavailability	1 Fixed	23.2 (20.7–26.1) ^f
Proportional error, %	6.91 (4.72–9.45)	
Additive error, mg/L	0.353 (0.303–0.408)	
Pregnancy effect on CL, %	+15.9 (9.75 to 21.9)	
INH effect on CL/F, L/hour, in <i>CYP2B6</i> fast metabolizers, %	-6.87 (-12.1 to -1.13)	
INH effect on CL/F, L/hour in <i>CYP2B6</i> intermediate and slow metabolizers, %	-13.4 (-17.3 to -9.06)	

%CV, percent coefficient of variation; CI, confidence interval; CL, clearance; CL_{int}, clearance intrinsic; f_u, unbound fraction of efavirenz in plasma; INH, isoniazid; K_a, first-order rate constant of INH absorption; MTT, absorption mean transit time; NN, number of absorption transit compartment; Q/F, apparent intercompartmental clearance for INH; Q_H, blood liver flow; V_c, apparent central volume of distribution for INH; V_p, apparent peripheral volume of distribution for INH.

The bold values represent significant covariates in the models.

^aThe 95% CIs were obtained with the Standardized Infection Ratio procedure.

^bVariability was modeled with log-normal distribution and is presented as an approximate percentage CV. ^cClearance parameters are allometrically scaled based on fat-free mass (typical value reported for 39 kg, which was the median fat-free mass weight of the study population). ^dVolume of distribution parameters are scaled based on weight (typical value reported for 67 kg, which was the median weight of the study population). ^eBetween subject variability. ^fBetween occasion variability.

more important for efavirenz clearance, but no significant effect was observed.

The model-predicted individual exposures are shown in **Figure 4**, where panels a and b stratify by *CYP2B6* genotype and pregnancy status, whereas panels c and d stratify by *CYP2B6* genotype and isoniazid co-administration. **Figure 4** shows that efavirenz varied among the three genotypes, and panels a and b show a decrease in exposures during pregnancy. The overall exposure of efavirenz was higher postpartum compared with pregnancy with median (interquartile range) postpartum AUC_{0–24} of 70.6 mg·h/L (47.9–118 mg·h/L), compared with 55.8 mg·h/L

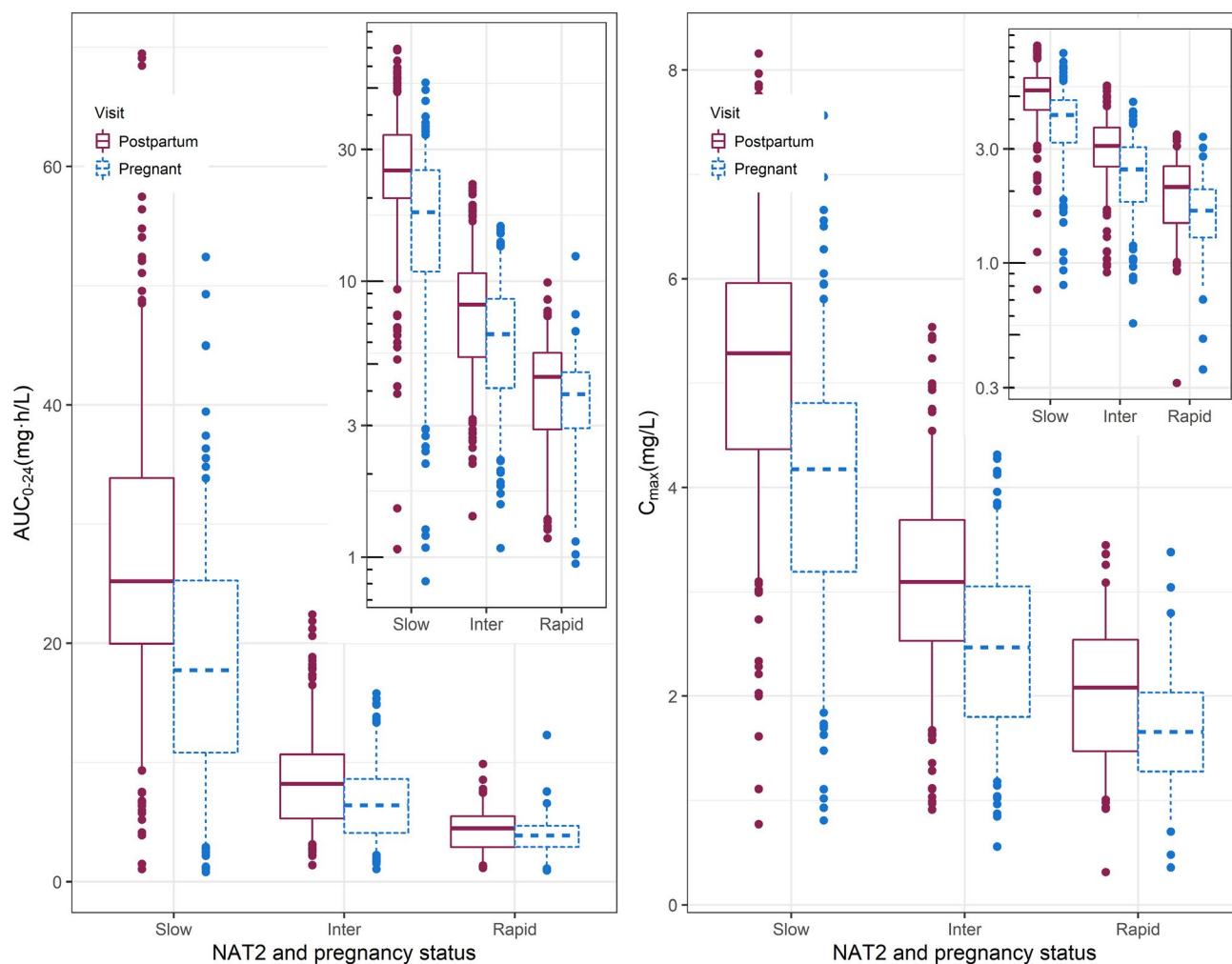


Figure 3 Isoniazid exposures stratified by NAT2 genotype and pregnancy. The box plot (with box representing median and interquartile range and whiskers the 5th–95th interval) summarizes isoniazid maximum concentration (C_{max}) on the right and 0–24-hour area under the concentration–time curve (AUC_{0-24}) on the left for the three genotypes (slow, intermediate, and rapid acetylator) for both the antepartum (red solid line) and postpartum (blue dashed lines) visit. AUC_{0-24} was calculated by integrating between the 0 and 24 hours after dosing time points. The inset panel shows the same values on the log-scale. [Colour figure can be viewed at wileyonlinelibrary.com]

(38.6–92.7 mg·h/L) antepartum. Concentrations at 12 hours postdosing (C_{12}) antepartum and postpartum were 2.18 mg·h/L (1.48–3.68 mg/L) and 2.69 mg·h/L (1.87–4.71 mg/L), respectively. Among normal *CYP2B6* metabolizers 13 women (9%) had postpartum efavirenz C_{12} of < 1 mg/L compared with 25 women (15%) antepartum.

We observed a DDI consisting of increased exposure of efavirenz in participants on concomitant isoniazid displayed in **Figure 4c,d**. The difference in exposure in the slow and intermediate metabolizers was more apparent than in the normal metabolizers resulting in high concentration in slow *CYP2B6* metabolizers. For example, choosing a threshold of 15 mg/L (similar to the upper quartile of C_{12} in *CYP2B6* slow metabolizers co-administered isoniazid in **Figure 4d**), among *CYP2B6* slow metabolizers in the absence of isoniazid, efavirenz C_{12} was > 15 mg/L in 4 individuals (9%), compared with 45 individuals (35%) with the presence of isoniazid. As a point of reference, a therapeutic range for plasma efavirenz concentrations of

1–4 mg/L has been suggested,³² although concentrations somewhat < 1 mg/L do not consistently predict treatment failure and concentrations > 4 mg/L do not consistently predict toxicity. The overall median (interquartile range) of AUC_{0-24} was 66.8 mg·h/L (45.2–114 mg·h/L) when isoniazid was present and 55.4 mg·h/L (39.7–98.1 mg·h/L) when absent, corresponding to a 1.2-fold increase in efavirenz AUC_{0-24} due to the presence of isoniazid. All four panels in **Figure 4** show that, whereas pregnancy and isoniazid affected efavirenz exposure, these effects were modest compared with *CYP2B6* genotype, which resulted in a 5-fold difference between slow to normal metabolizers.

DISCUSSION

We performed the largest study of its kind, assessing isoniazid and efavirenz PK, genetic polymorphisms, and DDIs in a geographic diverse population of pregnant and postpartum women living with HIV in high TB burden regions of the world. We show that pregnancy modestly increases the clearance of both isoniazid and

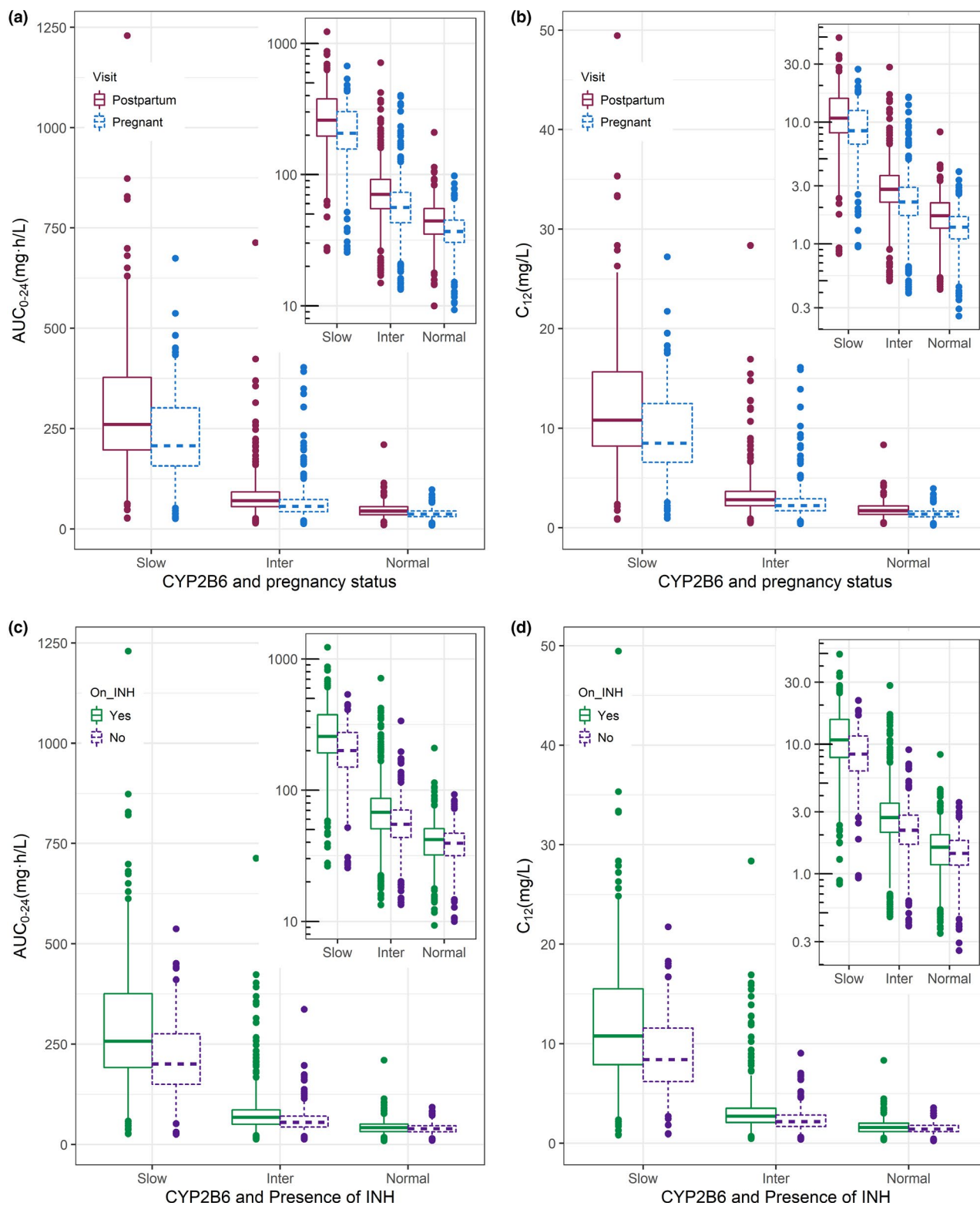


Figure 4 Efavirenz exposures stratified by CYP2B6 genotype, pregnancy and isoniazid co-administration. **(a, b)** Displays box plot (with box representing median and interquartile range and whiskers the 5th-95th interval) summarizing 0–24-hour area under the concentration-time curve (AUC_{0-24}) and concentrations at 12 hours postdosing (C_{12}), respectively, for the three genotypes (slow, intermediate, and normal metabolizer) stratified by pregnancy (solid line) and postpartum (dashed lines) visit. **(c, d)** Stratify AUC_{0-24} and C_{12} , respectively, using INH co-administration. [Colour figure can be viewed at wileyonlinelibrary.com]

efavirenz, therefore increasing the risk of low (and possibly subtherapeutic) drug exposure during pregnancy. Notably, although we also show that drug metabolizer genotypes profoundly affect plasma exposure of these two drugs, to an extent far greater than of pregnancy. For this reason, the risk of lower plasma drug exposure during pregnancy is likely greatest only in women who are *CYP2B6* normal metabolizers or *NAT2* rapid acetylator, because they clear the drug faster compared with the other genotypes and are already exposed to lower concentrations. Additionally, we report that efavirenz exposure is increased by isoniazid co-administration, especially in *CYP2B6* intermediate and slow metabolizers. Last, high concentrations observed in slow *CYP2B6* and *NAT2* genotype increases the risk of adverse effects.

The increase in clearance of isoniazid and efavirenz may be due to several physiological changes related to pregnancy (e.g., plasma albumin levels,⁶ bodyweight and composition, plasma volume,⁹ hepatic blood flow,⁹ and induction or inhibition of drug metabolizing enzymes). The pregnancy-induced increase in weight was accounted for in the model using allometric scaling, whereas no unbound concentration was captured, therefore, we could not confirm or disprove the effect of pregnancy on the protein-binding of efavirenz. Antepartum increase in hepatic blood,⁹ potentially increase the hepatic clearance of drugs with high hepatic extraction ratio, may be more apparent in low binding drugs like isoniazid compared with efavirenz. The other reason for changes in drug clearance during pregnancy is induction or inhibition of drug-metabolizing enzymes. Pregnancy increases the activity of *CYP2B6*⁷ and *CYP3A4*⁶ (major and minor route of efavirenz metabolism³³). Tsutsumi *et al.*⁸ observed a reduction in *NAT2* activity during pregnancy, but the reduction was clinically not significant. The reported clearance increase is modest compared with the large BSV in drug exposure due to host genetics. However, these changes may be of relevance for rapid/normal metabolizers, who already experience lower exposure, as further increase in clearance might increase their risk for subtherapeutic concentrations.

Similar to previous reports,^{10,12,34} we observed a DDI between isoniazid and efavirenz, as isoniazid decreases the clearance of efavirenz, particularly for intermediate and slow efavirenz metabolizers. This is thought to be due to isoniazid's inhibition of *CYP2A6*.³⁵ However, for individuals who are *CYP2B6* slow metabolizers, *CYP2A6* assumes increased importance in clearing efavirenz, so that *CYP2B6* slow metabolizers on isoniazid may have a higher risk of efavirenz toxicity. Our analysis did not detect any difference in isoniazid exposure among different ARTs (efavirenz-based, nevirapine-based, lopinavir/ritonavir-based, and atazanavir/ritonavir-based), which contrasts with previous reports.^{36,37} However, it may be that our analysis was not powered to detect these differences because 88% of the participants were on efavirenz-based ART.

Previously reported isoniazid exposures varied widely between studies. This might be due to effects of large BSV and between-occasion variability, different proportions of *NAT2* acetylator genotypes, different drug formulations, DDIs, and instability of isoniazid in plasma,³⁸ which makes PK studies of isoniazid somewhat challenging. This wide range is well summarized in a recent review by Daskapan *et al.*³⁹ Comparing our

results with previous reports on nonpregnant populations,^{39–41} we observed lower isoniazid exposures during pregnancy and postpartum visits, whereas the ratio in exposures between slow and rapid *NAT2* genotype was similar (clearance for rapid acetylators was observed to be 4-fold faster than those of slow acetylators). It is unclear if the relatively low concentrations we observed at both visits are due to efavirenz exposure, as most women were on efavirenz-based regimen. It is also possible that the effect of pregnancy on isoniazid clearance takes time to reverse fully, and women in the postpartum visit may also have clearance levels higher than the general (nonpregnant, adult) population. It has been previously discussed that using the postpartum period as a control to study effects of pregnancy may be suboptimal because of factors that include lactation and delayed reversal of pregnancy-related physiological changes.⁴² Efavirenz exposures in our study were in line with previous reports in nonpregnant individuals.^{33,43,44} Similarly, the ratio between normal and slow *CYP2B6* metabolizers exposures was similar, with a C_{12} ratio of 5.

In our study, we report genotype frequencies from a large cohort of patients with wide geographic representation. A frequency of *NAT2* slow acetylators above 50% worldwide has been reported.⁴⁵ Studies have shown greater than two-fold difference in the prevalence of *NAT2* slow acetylators in agriculturists (slow acetylators more prevalent) compared with pastoralists in central Asian population and sub-Saharan African populations.⁴⁶ A pooled analysis by Sabbagh *et al.*⁴⁵ shows the highest level of within-population diversity of *NAT2* genotype in Africans. In our study population, *NAT2* slow acetylators were most prevalent, followed by intermediate. Among CYP genes in humans, *CYP2B6* is one of the most polymorphic gene.⁴⁷ A meta-analysis by Zhou *et al.*⁴⁸ reported *CYP2B6* normal metabolizer status in 61%, 38%, 76%, 45%, and 58% in Europeans, Africans, East Asians, South Asians, and admixed Americans, respectively. In comparison, 65%, 65%, 31%, 66%, and 72% distribution of *CYP2A6* normal functioning gene were reported in Europeans, Africans, East Asians, South Asians, and admixed Americans, respectively. In the present study, intermediate followed by normal metabolizer *CYP2B6* genotypes were frequent in all countries, as were *CYP2A6* normal metabolizer genotypes.

Our study had some limitations, much of which we believe have been mitigated by use of a model-based approach analysis. Genotype data was not available in a subset of patients, 179 (21%) for isoniazid, and 173 (22%) for efavirenz, but this was addressed by imputation using a mixture model to assign individuals with missing information to a phenotype group. Blood draws during sparse sampling visits were scheduled for 2 hours after isoniazid dose. Because efavirenz is typically taken at night, this resulted in concentrations drawn 10–14 hours after efavirenz dose. Exact time of dose was not always reliably recorded for sparse sampling visits. For a portion of the study, timing of ART dosing was not recorded, so that information was imputed. Although imputed data cannot be expected to be fully accurate, our approach was feasible to implement and expected to be robust because most women were taking efavirenz in the evening and were sampled

the following morning, hence the slow terminal half-life (40–55 hours⁴⁹) and accumulation of efavirenz minimized the impact of imprecise dosing time information. The uncertainty around self-reported or imputed dosing times and sampling schedule chosen for the sparse data may be the reasons for high variability observed in the absorption parameters, but this had a minor effect in our analysis because the structural model was built using the intensive data which had rich and reliable information for the entire PK profile. Similarly, the method we used for the identification and exclusion of implausible outlier values prevented these few samples from affecting the development of the PK models.

In conclusion, our study showed modest reductions in isoniazid and efavirenz exposure during pregnancy compared with postpartum. Although the size of this effect is modest and unlikely to be of clinical significance for most patients, lower exposure may be important in normal *CYP2B6* and rapid *NAT2* acetylator, for whom the resulting concentrations may be sub-therapeutic, thus possibly leading to ineffective treatment and development of drug resistance. We also confirm the previously reported increased efavirenz exposure in individuals on concomitant isoniazid. This effect was overall modest but was most pronounced in *CYP2B6* intermediate and slow metabolizers, who already had higher efavirenz levels and may, therefore, be at higher risk of efavirenz-related toxicities.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICT OF INTEREST

All authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

K.G., P.D., A.G., D.H., C.L.W., and D.C. wrote the manuscript. A.G., D.H., R.B., G.M., L.A., K.M., S.B., A.W., T.R.S., G.H., P.J.P., N.C., and C.L.W. designed the research. L.W., J.N., C.L.W., C.O.M., T.C., D.H., R.B., N.C., G.M., L.A., K.M., S.B., T.V., L.S.C., G.R.M., A.V., B.T.M., L.A., R.B., N.N., V.R., E.K., M.M., V.C., M.N., T.M., F.T., A.H., K.S., B.Z., D.C., P.J.P., T.R.S., G.T., A.W., and A.G. performed the research. K.G., P.D., G.M., and L.A. analyzed the data.

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