J Antimicrob Chemother 2019; **74**: 3003–3010
doi:10.1093/jac/dkz298 Advance Access publication 12 July 2019

Journal of Antimicrobial Chemotherapy

Effect of patient genetics on etonogestrel pharmacokinetics when combined with efavirenz or nevirapine ART

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Received 29 January 2019; returned 3 April 2019; revised 10 June 2019; accepted 12 June 2019

Background: We previously demonstrated that etonogestrel concentrations were 82% lower in women using etonogestrel contraceptive implants plus efavirenz-based ART compared with women not receiving ART.

Objectives: To investigate the genetic contribution to this previously observed drug–drug interaction through studying SNPs in genes known to be involved in efavirenz, nevirapine or etonogestrel metabolism in the same group of women.

Patients and methods: Here, we present a secondary analysis evaluating SNPs involved in efavirenz, nevirapine and etonogestrel metabolism and associated etonogestrel pharmacokinetics among 57 women, 19 not receiving ART (control group), 19 receiving efavirenz- (600 mg daily) based ART and 19 receiving nevirapine- (200 mg twice daily) based ART. Associations between patient genotype and etonogestrel pharmacokinetic parameters were determined through univariate and multivariate linear regression. This study was registered at clinicaltrials.gov (NCT02082652).

Results: Within the control group, *CYP2B6* 983 T>C was associated with 27% higher etonogestrel C_{max} and 28% higher $AUC_{0-24weeks}$. In the efavirenz group *CYP2B6* 516 G>T was associated with 43% lower etonogestrel C_{min} and 34% lower $AUC_{0-24weeks}$. For participants receiving nevirapine, *NR1I2* 63396 C>T was associated with 39% lower etonogestrel C_{min} and 37% lower $AUC_{0-24weeks}$.

Conclusions: This study demonstrates the influence of pharmacogenetics on the extent of drug–drug interactions between etonogestrel and efavirenz- or nevirapine-based ART. Efavirenz plus the etonogestrel contraceptive implant results in a detrimental drug–drug interaction irrespective of patient genetics, which is worsened in women possessing variant alleles for these *CYP2B6* SNPs.

Introduction

Within sub-Saharan Africa, 80% of new HIV cases in adolescents are among girls.[1] More highly effective contraceptive options are needed to support the needs of this growing demographic and to help reduce the incidence of mother to child transmission. The etonogestrel subdermal implant is an effective contraceptive method recommended by the WHO.[2] The antiretroviral drug efavirenz is a first-line HIV medication also recommended by the WHO; however, concomitant use of efavirenz and the etonogestrel implant results in a significant drug–drug interaction resulting in reduced etonogestrel exposure and unintended pregnancies.[3–6]

We previously demonstrated etonogestrel concentrations to be 82% lower in Ugandan women receiving efavirenz-based ART compared with women not receiving ART, while nevirapine-based ART did not result in a significant drug–drug interaction with etonogestrel.[6] Additionally our group has previously reported an association between *CYP2B6* SNPs with alterations in the pharmacokinetics of levonorgestrel released from a subdermal implant when prescribed concomitantly with efavirenz or nevirapine.[7] Etonogestrel and levonorgestrel are both approved for use as progestin-only contraceptive implants and have similar metabolism pathways, both being primarily metabolized by CYP3A4.[8,9] We sought to investigate potential associations between SNPs involved in efavirenz, nevirapine and etonogestrel metabolism with etonogestrel pharmacokinetics in the same group of women, including SNPs within the *CYP2B6*, *NR1I2*, *CYP3A4* and *ABCB1* genes.

NR1I2 encodes the pregnane X receptor (PXR) responsible for regulation of expression of multiple enzymes including CYP3A4.[8,10] *ABCB1* SNPs have previously been associated with alterations in efavirenz plasma concentrations.[11] *CYP2B6* SNPs have been linked with alterations in efavirenz and nevirapine pharmacokinetics in a multitude of studies within patients of different ethnicities.[12–20] Efavirenz is an inducer of CYP3A4 activity, resulting in enhanced systemic clearance of co-administered CYP3A4 substrates.[21–23] Furthermore, efavirenz activates PXR, which is responsible for transcriptional regulation of CYP3A4, in a dose-dependent manner *in vitro*.[23] We hypothesize that alterations in efavirenz or nevirapine concentrations, caused by SNPs within associated genes, would have a secondary effect of altering etonogestrel metabolism, through the antiretroviral drug altering the activity of enzymes involved in the metabolism of etonogestrel.[21–23]

Patients and methods

Ethical approval

All study procedures occurred at the Infectious Disease Institute (IDI) in Kampala, Uganda and were approved by the University of Pittsburgh (PRO14010195), the Joint Clinical Research Centre and Uganda National Council of Science and Technology (HS 1618). This study followed the Declaration of Helsinki and was registered at clinicaltrials.gov (NCT02082652).

Study design and cohort

Full information on the study design and participants has been described previously by Chappell *et al.*[6] In brief, this pharmacogenetics substudy included 57 of the 60 Ugandan women enrolled into the parent study, 19 receiving nevirapine- (200 mg twice daily) and 19 receiving efavirenz- (600 mg daily) based ART for HIV treatment. Statistical analysis was also completed for the 19 participants within the antiretroviral-naive (control) arm of the study to assess the influence of pharmacogenetics in the absence of concomitant ART. Exclusionary criteria included, but were not limited to, HIV RNA >400 copies/mL in participants receiving ART, CD4+ cell count <350 cells/mm^3 in the antiretroviral-naive group and coadministration of medication contraindicated for use with etonogestrel, efavirenz or nevirapine within the respective groups. In light of the growing number of cases of observed pregnancies in women receiving efavirenz who have a contraceptive implant, participants in the efavirenz group had a copper intrauterine device inserted prior to study initiation to minimize risk of unintended pregnancy in the event of etonogestrel contraceptive failure.

Sample and data collection

Study visits occurred at 1, 4, 12 and 24 weeks after implant placement. Blood samples were taken in order to determine the etonogestrel concentration at each study visit. For efavirenz and nevirapine, a single timed blood sample was taken twice before implant insertion and 4, 12 and 24 weeks after implant insertion. For nevirapine sampling, blood was drawn 11–13 h after the participant's last nevirapine dose. For efavirenz, sampling was completed 12–14 h after the last efavirenz dose. Etonogestrel concentrations were quantified from plasma through week 24 after etonogestrel implant placement, using HPLC-MS.[24] For nevirapine and efavirenz quantification, HPLC was performed utilizing validated methods.[25,26] The pharmacokinetic parameters included in this study were AUC from entry to week 24 ($AUC_{0–24weeks}$), C_{max}, T_{max} and C_{min}. C_{max} and C_{min} represent the highest and lowest concentrations observed over the entire study period. AUC was calculated using the trapezoidal rule (Phoenix WinNonlin, Certara®).

Genotyping

Patient DNA was extracted from whole blood through use of the manufacturer's protocol (E.Z.N.A Blood DNA Mini Kit; Omega Bio-tek, Norcross, GA, USA). Genotyping was completed using a real-time allelic discrimination PCR assay on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Hercules, CA, USA). The PCR protocol involved denaturation at 95°C for 10 min, followed by 50 cycles of amplification at 92°C for 15 s and annealing at 60°C for 1 min 30 s. Samples were genotyped for the following SNPs utilizing Taqman assays: *CYP2B6* 516 G>T (rs3745274), 983 T>C (rs28399499) and 15582 C>T (rs4803419), *NR1I2* 63396 C>T (rs2472677), *CYP3A4* 392 G>A (rs2740574), *ABCB1* 4036 A>G (rs3842) and 3435 C>T (rs1045642) using Taqman Genotyping Master mix and corresponding Taqman Genotyping assays purchased from Thermo Fisher Scientific (Wilmington, DE, USA). Opticon Monitor v.3.1 software (Bio-Rad Laboratories) was used to obtain allelic discrimination plots and identify genotypes.

Statistical analysis

Compliance for each SNP with Hardy–Weinberg equilibrium was tested through previously outlined methods.[27] Genotypes were coded for regression analyses as 0=homozygous common allele, 1=heterozygous and 2=homozygous variant allele. Categorical variables were described using relative frequencies; continuous variables were described using the median and IQR. The Shapiro–Wilk test was used to test for normality, with $P \leq 0.05$ considered as statistically significant. Associations between patient genotype and etonogestrel pharmacokinetic parameters were determined through univariate and multivariate linear regression. A univariate analysis through linear enter regression was carried out in order to identify independent variables associated with etonogestrel pharmacokinetic parameters within each study group. Variables with $P \leq 0.2$ for the univariate analysis were carried through to a linear backwards multivariate analysis, with $P \leq 0.05$ considered statistically significant. All statistical analyses were carried out using IBM SPSS Statistics v.24 (IBM Armonk, NY, USA). All charts were produced using GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA).

Results

Etonogestrel, efavirenz and nevirapine pharmacokinetics

In total, 57 women living with HIV were included in the analysis, 19 receiving efavirenz, 19 receiving nevirapine and 19 not receiving ART (control group). All genotypes and patient characteristics are summarized in Table 1. The median (IQR) age and weight of all participants was 28 years (25–34 years) and 57 kg (50–69 kg). All SNPs were in Hardy–Weinberg equilibrium, with the exception of *ABCB1* 4036 A>G, which compromises interpretation of this SNP. Statistically significant univariate and multivariate regression analysis results of each group are presented in Table 2. Full regression analysis results are shown in Table S1 (available as Supplementary data at *JAC* Online).

Control group

Within the control group, *CYP2B6* 983 T>C was significantly associated with higher $\log_{10}$ etonogestrel C_{max} (P=0.013, β=0.193) and higher $\log_{10}$ etonogestrel $AUC_{0–24weeks}$ (P=0.011, β=0.188); equivalent to 10% higher etonogestrel C_{max} and 76% higher etonogestrel $AUC_{0–24weeks}$ in participants heterozygous CT compared with those homozygous TT. *CYP3A4* 392 G>A was also significantly

Table 1. Characteristics and genotype frequencies of the study participants at entry

	Total (n=57)			Control group (n=19)			Efavirenz group (n=19)			Nevirapine group (n=19)		
Characteristics												
age (years)	28 (25–34)			27 (24–30)			29 (23–35)			32 (28–35)		
height (cm)	160 (155–163)			160 (154–165)			157 (150–165)			161 (155–164)		
weight (kg)	57 (50–69)			62 (49–78)			56 (48–64)			56 (51–82)		
CD4 count (cells/mm^3)	624 (441–1050)			832 (624–1483)			449 (274–1072)			544 (428–853)		
Genotype frequencies												
CYP2B6 516G>T (rs3745274) (%)	GG	GT	TT	GG	GT	TT	GG	GT	TT	GG	GT	TT
	40	53	7	57	37	6	32	58	11	32	63	5
CYP2B6 983T>C (rs28399499) (%)	TT	CT	CC	TT	CT	CC	TT	CT	CC	TT	CT	CC
	82	18	0	84	16	0	84	16	0	79	21	0
CYP2B6 15582C>T (rs4803419) (%)	CC	CT	TT	CC	CT	TT	CC	CT	TT	CC	CT	TT
	89	11	0	89	11	0	95	5	0	84	16	0
NR1I2 63396C>T (rs2472677) (%)	CC	CT	TT	CC	CT	TT	CC	CT	TT	CC	CT	TT
	39	44	17	37	42	21	37	47	16	47	42	11
CYP3A4 392G>A (rs2740574) (%)	GG	AG	AA	GG	AG	AA	GG	AG	AA	GG	AG	AA
	47	44	9	47	37	16	42	58	0	53	37	10
ABCB1 4036A>G (rs3842) (%)	AA	AG	GG	AA	AG	GG	AA	AG	GG	AA	AG	GG
	70	14	16	84	11	5	58	21	21	68	11	21
ABCB1 3435C>T (rs1045642) (%)	CC	CT	TT	CC	CT	TT	CC	CT	TT	CC	CT	TT
	74	26	0	68	32	0	68	32	0	84	16	0

Values are shown as median (IQR) and percentage of population.

associated with higher $\log_{10}$ etonogestrel C_{max} (P=0.028, β=0.083) and higher $\log_{10}$ etonogestrel $AUC_{0–24weeks}$ (P=0.034, β=0.076); equivalent to 64% higher etonogestrel C_{max} and 63% higher etonogestrel $AUC_{0–24weeks}$ in participants homozygous G compared with those homozygous A (Tables 2 and 3).

Efavirenz group

As shown in Table 2 and Figure 1, *CYP2B6* 516 G>T was associated with a lower $\log_{10}$ etonogestrel C_{min} (P=0.003, β=−0.102) and lower $\log_{10}$ etonogestrel $AUC_{0–24weeks}$ (P=0.008, β=−0.106) for participants receiving efavirenz. This equates to a 43% difference in etonogestrel C_{min} and a 34% difference in etonogestrel $AUC_{0–24weeks}$ between participants with homozygous G and homozygous T genotypes for *CYP2B6* 516 G>T, respectively (see Table 3).

CYP2B6 983 T>C was associated with lower $\log_{10}$ etonogestrel C_{max} (P=0.003, β=−0.237) and lower $\log_{10}$ etonogestrel $AUC_{0–24weeks}$ (P=0.016, β=−0.158), which equates to a 37% difference in etonogestrel C_{max} and a 20% difference in etonogestrel $AUC_{0–24weeks}$ between participants who were homozygous T and heterozygous CT for *CYP2B6* 983 T>C when prescribed efavirenz alongside the etonogestrel contraceptive implant (see Tables 2, 3 and Figure 1).

Based on prior data, an etonogestrel concentration of 90 pg/mL is the minimum concentration required to suppress ovulation.[6,28] In the context of the two SNPs associated with changes in etonogestrel exposure in the efavirenz group, we observed that the median etonogestrel concentration in all participants, regardless of genotype, fell below this concentration at all visits after the week 4 visit (Table 4). Further, participants who were homozygous (TT) or heterozygous (GT) for *CYP2B6* 516 G>T and those heterozygous CT for *CYP2B6* 983 T>C had a median concentration below 90 pg/mL by the week 4 visit.

As anticipated, efavirenz plasma concentration ($C_{12–14h}$) was 76% higher in participants homozygous T for *CYP2B6* 516 G>T and 69% higher in participants heterozygous CT for *CYP2B6* 983 T>C compared with participants who were homozygous T (Table 3).

Nevirapine group

For participants on nevirapine treatment, *NRI12* 63396 C>T was associated with lower $\log_{10}$ etonogestrel C_{min} (P=0.010, β=−0.091) and lower $\log_{10}$ etonogestrel $AUC_{0–24weeks}$ (P <0.001, β=−0.013); equivalent to 39% lower etonogestrel C_{min} and 37% lower etonogestrel $AUC_{0–24weeks}$ in participants homozygous TT compared with those homozygous CC. *CYP2B6* 983 T>C was associated with higher $\log_{10}$ etonogestrel C_{max} (P=0.013, β=0.187), which equates to a etonogestrel C_{max} difference of 41% between homozygous T and heterozygous CT participants. *CYP3A4* 392 G>A was associated with higher $\log_{10}$ etonogestrel $AUC_{0–24weeks}$ (P=0.004, β=0.096), which equates to an 18% difference in $\log_{10}$ etonogestrel $AUC_{0–24weeks}$ between homozygous G and homozygous A participants (Tables 2 and 3).

Nevirapine median plasma concentration ($C_{12–14h}$) was 7% lower in participants homozygous T for *NRI12* 63396 C>T compared with participants homozygous C, and 18% higher in participants heterozygous for *CYP2B6* 983 T>C compared with participants homozygous T. Furthermore, for participants homozygous A for *CYP3A4* 392 G>A, nevirapine plasma concentration ($C_{12–14h}$) was 10% higher than in participants homozygous G (Table 3).

Table 2. Statistically significant results from univariate and multivariate linear regression analysis within each study group

	Univariate linear regression			Multivariate linear regression		
	P	β (95% CI)	r^2	*P*	β (95% CI)	r^2
Efavirenz group						
$\log_{10}$ ENG C_{max}						
CYP2B6 516G>T (rs3745274)	0.135	−0.085 (−0.2, 0.0)	0.126			
***CYP2B6* 983T>C (rs28399499)**	0.014	−0.222 (−0.4,−0.5)	0.307	**0.003**	**−0.237 (−0.4, 0.1)**	**0.518**
CYP2B6 15582C>T (rs4803419)	0.070	−0.277 (−0.6, 0.3)	0.180			
ABCB1 4036A>G (rs3842)	0.110	0.068 (0.0, 0.2)	0.144			
ENG T_{max}						
$\log_{10}$ weight ($\log_{10}$ kg)	0.199	3.005 (−1.7, 7.8)	0.095			
***CYP2B6* 516G>T (rs3745274)**	0.045	0.507 (0.0, 1.0)	0.216	**0.045**	**0.507 (0.0, 1.0)**	**0.216**
$\log_{10}$ ENG C_{min}						
***CYP2B6* 516G>T (rs3745274)**	0.003	−0.102 (−0.2, 0.0)	0.423	**0.003**	**−0.102 (−0.2, 0.0)**	**0.423**
$\log_{10}$ ENG $AUC_{0-24weeks}$						
***CYP2B6* 516G>T (rs3745274)**	0.028	−0.098 (−0.2, 0.0)	0.255	**0.008**	**−0.106 (−0.2, 0.0)**	**0.487**
***CYP2B6* 983T>C (rs28399499)**	0.062	−0.142 (−0.3, 0.0)	0.190	**0.016**	**−0.158 (−0.3, 0.0)**	**0.487**
Nevirapine group						
$\log_{10}$ ENG C_{max}						
***CYP2B6* 983T>C (rs28399499)**	0.013	0.187 (0.0, 0.3)	0.313	**0.013**	**0.187 (0.0, 0.3)**	**0.313**
NR1I2 63396C>T (rs2472677)	0.058	−0.091 (−0.2, 0.0)	0.196			
$\log_{10}$ ENG C_{min}						
CYP2B6 983T>C (rs28399499)	0.062	0.114 (0.0, 0.2)	0.190			
***NR1I2* 63396C>T (rs2472677)**	0.010	−0.091 (−0.2, 0.0)	0.329	**0.010**	**−0.091 (−0.2, 0.0)**	**0.028**
$\log_{10}$ ENG $AUC_{0-24weeks}$						
CYP2B6 983T>C (rs28399499)	0.080	0.125 (0.0, 0.3)	0.170			
***CYP3A4* 392G>A (rs2740574)**	0.154	0.063 (−0.2, 0.0)	0.116	**0.004**	**0.096 (−0.2, 0.0)**	**0.643**
***NR1I2* 63396C>T (rs2472677)**	0.004	−0.116 (−0.2, 0.0)	0.388	**<0.001**	**−0.139 (−0.2,−0.1)**	**0.643**
Control group						
$\log_{10}$ ENG C_{max}						
***CYP2B6* 983T>C (rs28399499)**	0.053	0.159 (0.0, 0.3)	0.203	**0.013**	**0.193 (0.0, 0.3)**	**0.416**
***CYP3A4* 392G>A (rs2740574)**	0.133	0.063 (0.0, 0.1)	0.128	**0.028**	**0.083 (0.0, 0.2)**	**0.416**
$\log_{10}$ ENG $AUC_{0-24weeks}$						
***CYP2B6* 983T>C (rs28399499)**	0.043	0.156 (0.0, 0.3)	0.219	**0.011**	**0.188 (0.0, 0.3)**	**0.415**
***CYP3A4* 392G>A (rs2740574)**	0.160	0.056 (0.0, 0.1)	0.113	**0.034**	**0.076 (0.0, 0.1)**	**0.415**

ENG, etonogestrel.
Univariate linear regression ($P \leq 0.2$) completed, all statistically significant results then carried through to multivariate linear regression analysis ($P \leq 0.05$). All statistically significant variables from multivariate linear regression are shown in bold.

Discussion

This study demonstrates associations between genetic variations in *CYP2B6* 516 G>T and 983 T>C with multiple pharmacokinetic parameters of etonogestrel in women treated with efavirenz using etonogestrel contraceptive implants. Our group has previously described a genetic association between SNPs in *CYP2B6* and lower pharmacokinetics of levonorgestrel given as a subdermal implant in women receiving efavirenz.[7] Here we describe 33% lower etonogestrel $AUC_{0-24weeks}$ within homozygous T participants compared with homozygous G participants for *CYP2B6* 516 G>T. For levonorgestrel $AUC_{0-24weeks}$, 64% lower results were observed for homozygous T participants compared with homozygous G for *CYP2B6* 516 G>T.[7] Furthermore, 20% lower etonogestrel $AUC_{0-24weeks}$ was seen between homozygous C and heterozygous CT participants for *CYP2B6* 983 T>C,[7] similar to the 23% lower levonorgestrel $AUC_{0-24weeks}$ observed between these genotypes in our previous study. Greater reductions were seen in etonogestrel pharmacokinetic exposure in the presence of *CYP2B6* SNPs associated with reduced efavirenz metabolism. This finding may be explained by higher concentrations of efavirenz resulting in increased CYP3A4 activity and expression that is known to enhance elimination of etonogestrel. This is supported by a previous study of the effect of varying concentrations of efavirenz on CYP3A4 activity that demonstrated a dose-dependent induction of CYP3A4 by efavirenz.[22] Furthermore, *CYP2B6* 516 G>T and 983 T>C have been shown to result in reduced *CYP2B6* expression.[18,29]

In previous work, we reported an association between *NRI12* 63396 C>T and higher levonorgestrel T_{max}. Also, we observed an association between *CYP2B6* 516 G>T and higher levonorgestrel C_{min} and C_{max}.[7] The consistent findings of these two studies

Table 3. Etonogestrel, efavirenz and nevirapine pharmacokinetic parameters shown as median (IQR), summarized by associated *CYP2B6*, *NR1I2* or *CYP3A4* genotype

	CYP2B6 516G>T (rs3745274)			*CYP2B6* 983T>C (rs28399499)			*NR1I2* 63396C>T (rs2472677)			*CYP3A4* 392G>A (rs2740574)		
	GG	GT	TT	TT	CT	CC	CC	CT	TT	GG	AG	AA
Allele frequency												
EFV group	6	11	2	16	3	0	7	9	3	8	11	0
NVP group	6	12	1	15	4	0	9	8	2	10	7	2
control group	11	7	1	16	3	0	7	8	4	9	7	3
ENG C_{max} (pg/mL)												
EFV group	160 (158–185)	133 (102–207)	97 (85–109)	148 (109–207)	93 (75–102)	–	102 (101–207)	136 (85–220)	148 (114–178)	148 (108–213)	114 (101–185)	–
NVP group	585 (533–895)	514 (489–781)	693	533 (498–705)	913 (701–1124)	–	701 (498–1124)	585 (514–705)	480 (460–500)	502 (489–701)	650 (585–895)	674 (500–847)
control group	840 (756–959)	868 (685–974)	527	840 (650–971)	1157 (959–1196	–	949 (922–1022)	840 (756–971)	667.5 (650–685)	756 (527–949)	959 (868–972)	922 (840–974)
ENG T_{max} (week)												
EFV group	1 (1–1)	1 (1–1)	2.5 (1–4)	1 (1–1)	1 (1–1)	–	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	–
NVP group	1 (1–1)	1 (1–1)	1	1 (1–1)	1 (1–1)	–	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)
control group	1 (1–1)	1 (1–1)	1	1 (1–1)	1 (1–1)	–	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)	1 (1–1)
ENG C_{min} (pg/mL)												
EFV group	81 (63–84)	65 (57–76)	46 (40–52)	67 (53–81)	60 (57–62)	–	57 (53–71)	158 (133–185)	57 (53–104)	60 (53–85)	67 (57–80)	–
NVP group	302 (269–461)	514 (489–781)	368	343 (280–375)	438 (369–507)	–	369 (324–461)	349 (302–394)	222 (174–269)	333 (280–404)	354 (302–461)	322 (269–375)
control group	321 (281–427)	393 (249–513)	297	300 (249–420)	480 (407–509)	–	268 (249–427)	374 (297–513)	318 (243–393)	297 (209–480)	407 (374–513)	300 (249–427)
ENG $AUC_{0-24weeks}$ (pg·week/mL)												
EFV group	2052 (1679–2669)	1664 (1537–2146)	1364 (968–1760)	1760 (1587–2405)	1405 (1142–1597)	–	1537 (1405–2405)	1978 (1679–2146)	1587 (1571–2669)	1679 (1587–2669)	1664 (1405–2148)	–
NVP group	9048 (8492–16217)	9902 (8095–11299)	13420	9805 (8095–11299)	13185 (11540–14829)	–	11540 (9902–15145)	8492 (8088–11299)	7179 (5311–9048)	9805 (7778–11540)	10978 (8492–16217)	12096 (9048–15145)
control group	10492.5 (9753.5–14300)	10765 (10024.5–14740.5)	7855	10332 (8636–11794.5)	14300 (13448.5–16205.5)	–	10765 (10024.5–14300)	10492.5 (9753.5–15821.5)	9484 (8636–10332)	10185.5 (6112–13448.5)	11794.5 (10332–14740.5)	10765 (9753.5–14478)
EFV C_{12-14h} (mg/L)	2.1 (2.0–2.7)	3.2 (2.9–6.6)	8.9 (8.1–9.7)	2.9 (2.5–4.3)	9.3 (7.05–11.4)	–	3.0 (2.9–6.6)	2.9 (2.0–4.9)	3.3 (2.7–6.6)	2.7 (2.1–4.9)	3.2 (2.7–9.3)	–
NVP C_{11-13h} (mg/L)	5.9 (5.6–7.1)	6.4 (4.8–7.9)	11.0	6.2 (4.7–7.1)	7.6 (7.4–7.8)	–	6.5 (4.7–7.9)	6.2 (5.9–11.0)	6.0 (4.8–7.1)	5.6 (4.0–7.9)	6.3 (5.9–11.0)	6.2 (7.3–5.2)

ENG, etonogestrel; EFV, efavirenz; NVP, nevirapine.
Efavirenz C_{12-14h} (mg/L) and nevirapine C_{11-13h} (mg/L) determined from individual participant's geometric mean value calculated from concentration measured at study entry and weeks 1, 4, 12, 24 and 48 summarized for the group as median (IQR).

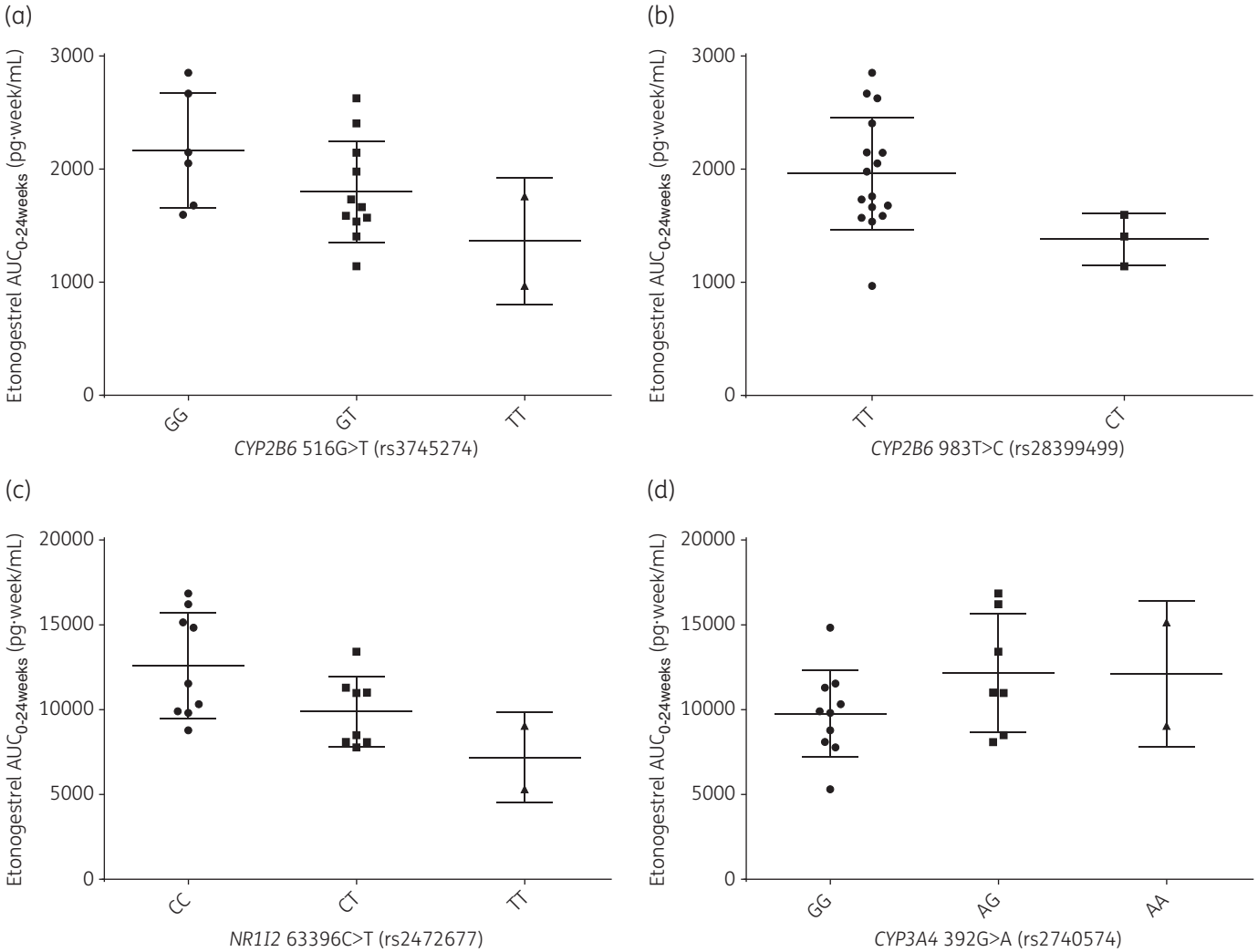

Figure 1. Etonogestrel pharmacokinetics compared by statistically significant genotype within the efavirenz (a and b) and nevirapine (c and d) groups. Data are represented by mean (SD) and compared by genotype for each of the SNPs significantly associated with etonogestrel $AUC_{0-24weeks}$ found through multivariate analysis (P=0.05) within the efavirenz group (a and b) and the nevirapine group (c and d).

Table 4. Etonogestrel concentration per week of study summarized by significant *CYP2B6* SNP genotype within the efavirenz group

	Etonogestrel concentration (pg/mL)			
	week 1	week 4	week 12	week 24
CYP2B6 516G>T (rs3745274)				
GG (n=6)	160 (158–185)	92 (79–107)	74 (68–110)	81 (63–84)
GT (n=11)	114 (101–220)	78 (58–135)	53 (48–83)	62 (53–80)
TT (n=2)	92 (85–99)	72.5 (36–109)	50.5 (36–65)	46 (40–52)
CYP2B6 983T>C (rs28399499)				
TT (n=16)	148 (108–207)	92 (78–121)	64 (53–83)	67 (53–81)
CT (n=3)	94 (75–102)	58 (48–74)	54 (32–68)	60 (57–62)
CC (n=0)	–	–	–	–

Values are shown as median (IQR)

strengthen the evidence base in support of a genetic contribution to the drug–drug interaction between contraceptive hormonal treatments and efavirenz- or nevirapine-based ART. Taken together, these studies imply that greater risk of contraceptive failure exists in women with variant alleles for *CYP2B6* SNPs who receive efavirenz and levonorgestrel- or etonogestrel-based contraceptive implants.

Within the nevirapine group, *NRI12* 63396 C>T, *CYP3A4* 392 G>A and *CYP2B6* 983 T>C were associated with alterations in etonogestrel pharmacokinetics. The association of *CYP3A4* 392 G>A with higher $\log_{10}$ etonogestrel $AUC_{0-24weeks}$ is a novel finding in this study. *CYP3A4* 392 G>A is found in the promoter region of *CYP3A4*.[30] The presence of this SNP alters the transcription binding site of the promoter region, where it is hypothesized to effect protein binding and thus reduce gene expression.[30] This mechanism of action may explain the observed relationship, as reduced expression of CYP3A4 results in lesser metabolism of etonogestrel, irrespective of the presence of nevirapine, as demonstrated within HIV-positive women using a etonogestrel contraceptive implant without ART in the control group (Tables 2 and 3), where *CYP3A4* 392 G>A was associated with higher $\log_{10}$ etonogestrel $AUC_{0-24weeks.}$ The relationship between *CYP2B6* 983 T>C and higher etonogestrel C_{max} contradicts that observed within the efavirenz group and is surprising given that nevirapine is an inducer of CYP3A4.[31] However, this result mirrors the findings within the control group, where *CYP2B6* 983 T>C was associated with a 27% higher etonogestrel C_{max} between TT and CT genotype patients. Additionally these findings mirror that observed within our levonorgestrel study, where *CYP2B6* 516 G>T was significantly associated with higher levonorgestrel C_{min} and C_{max} within the nevirapine group.[7] While these consistent findings support the legitimacy of an association, a biological mechanism for this interaction is yet to be elucidated. The contradictory nature of the relationship between nevirapine pharmacokinetics and *CYP2B6* 983 T>C has been discussed previously, and a larger cohort study would be required to confirm the strength of the observations within our two studies.[32]

Notably, due to the extent of the interaction between efavirenz and etonogestrel observed (82% lower etonogestrel exposure), the median concentration of etonogestrel for all participants, irrespective of *CYP2B6* genotype, fell below the concentration desired to suppress ovulation after week 4. Clinical studies are currently under way to determine the suitability of a dose alteration of etonogestrel or levonorgestrel to overcome this observed drug–drug interaction in patients receiving efavirenz. These studies are in the form of patients receiving either two etonogestrel (132 mg) or two levonorgestrel (300 mg) implants at once: clinical trials.gov registration numbers NCT03282799 and NCT02722421, respectively.[33,34] The findings of these studies will be useful in determining if this approach can mitigate the interaction observed between efavirenz and progestin-based implants.

Use of physiologically based pharmacokinetic modelling to examine the effect of a reduction in efavirenz dose (600 to 400 mg) on the previously observed interaction between the 150 mg levonorgestrel subdermal implant and efavirenz predicted that efavirenz dose reduction would not fully mitigate the effect of efavirenz co-administration.[35,36] A similar investigation would be of utility for etonogestrel, given the greater degree of variation in etonogestrel concentrations observed between week 1 and week 24 when prescribed concomitantly with efavirenz (geometric mean at week 24=66 pg/mL: a 51% reduction in etonogestrel concentration from study week 1) compared with that seen for levonorgestrel prescribed alongside efavirenz at study week 24 (geometric mean at week 24=280 pg/mL: 31% reduction in levonorgestrel concentration from study week 1).[6,35]

Our study included only Ugandan women of African ancestry, with the significant SNPs in the efavirenz group found predominantly in African patients.[32] Further pharmacogenetics studies in women of different ethnicities would be necessary to understand if women of particular ethnicities are at higher risk of contraceptive implant failure compared with others. Future studies would benefit from recruitment of a larger sample size, given the limited number of patients within the statistically significant populations and that *ABCB1* 4036 A>G was not in Hardy–Weinberg equilibrium.

Overall, drug–drug interactions between hormonal contraceptive implants and antiretroviral drugs may significantly compromise contraceptive efficacy in HIV-positive women and limit clinical treatment options in resource-constrained settings. In our participants receiving efavirenz, a cumulative effect of the *CYP2B6* SNP variant alleles on etonogestrel concentrations was observed throughout the study even though CYP2B6 is not involved in etonogestrel metabolism. This study demonstrates the influence of patient genetics on the pharmacokinetic exposure of contraceptive hormones mediated via a drug–drug interaction.

Acknowledgements

We thank the patients, staff and administration of the Infectious Diseases Institute, Makerere University, Kampala, Uganda, for their collaboration and support.

Funding

This work was supported by the National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development under award number 1R01HD085887 (Principal Investigator K. K. S.) and the Society of Family Planning Research Fund grant #SFPRF14-12.

Transparency declarations

C. A. C. is receiving research funding from Gilead Sciences and Merck though the Magee-Womens Research Institute and has served as a consultant for Gilead Sciences. B. A. C. was on a Merck contraceptive advisory board and has received research funding through Medicines360 and Merck, all managed by the Magee-Womens Research Institute. M. S. reports grants from ViiV and Janssen, outside the submitted work. A. O. has received research funding from Merck, AstraZeneca, Pfizer, ViiV and Janssen, has consulted for Merck and ViiV, and is also a coinventor of patents relating to nanotechnology-based drug delivery systems. M. L. has received research funding from ViiV and Janssen. All other authors: none to declare.

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Society of Family Planning Research Fund.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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