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CYP2B6 Variants Alter Etonogestrel Pharmacokinetics When Combined With Efavirenz

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INTRODUCTION

- The etonogestrel (ENG) implant is an effective hormonal contraceptive method commonly used in sub-Saharan Africa.
- Drug-drug interactions between contraceptive progestins and antiretrovirals, specifically efavirenz (EFV), continue to be a significant barrier to contraceptive use among women living with HIV.
- ENG concentrations were previously demonstrated to be 82% lower at week 24 in women receiving efavirenz (EFV) based antiretroviral therapy (ART) compared to women not receiving ART. (1)
- We investigated a genetic contribution to this previously observed drug-drug interaction through studying the following SNPs in the same group of women: CYP2B6 516G>T, 983T>C and 15582C>T, CYP3A4 293A>G, NR1I2 63396C>T, ABCB1 4036A>G and 3435C>T.
- CYP2B6 is the primary metaboliser of EFV and CYP3A4 is the primary metabolizer of ENG. NR1I2 encodes the pregnane X receptor, a transcriptional regulator of multiple CYPs including CYP2B6 and CYP3A4. ABCB1 encodes the P-glycoprotein receptor a membrane bound transporter of many endogenous substrates and xenobiotics, including EFV.

METHODS

Ethics: All study procedures 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 Population: 19 patients receiving EFV (600mg daily) based ART. Exclusionary criteria included but was not limited to HIV RNA >400 copies/mL and participants receiving any medication that was contraindicated for use with ENG or EFV. Participants in the EFV group had a copper intrauterine device inserted prior to study initiation to minimise the risk of unintended pregnancy in the event of ENG contraceptive failure.

Sample Collection: Study visits occurred at 1, 4, 12 and 24 weeks post implant placement. Blood samples were taken in order to determine ENG concentration at each study visit. For EFV a single timed blood sample was taken twice before implant insertion and 4, 12 and 24 weeks post implant insertion.

Pharmacokinetic Parameters: Pharmacokinetic parameters included in this analysis were area under the concentration time curve from entry to 24 weeks for ENG ($AUC_{0\text{-}24wk}$), maximum ENG concentration (C_{max}) and minimum ENG concentration (C_{min}). AUC was calculated using the trapezoidal rule. EFV concentrations were taken 12-14 hours post dose ($C_{12\text{-}14h}$).

ENG/EFV Concentration Quantification: ENG concentrations were quantified using HPLC/mass spectrometry, EFV concentrations were quantified using HPLC, utilising validated methods. (2)(3)

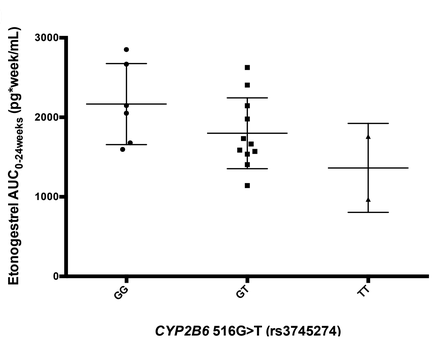
Genotyping: Genotyping was performed by allelic discrimination real-time PCR using TaqMan® SNP Genotyping assays for selected candidate SNPs.

Statistical Analysis: Univariate linear regression analysis was conducted with a $P \leq 0.20$ classed as significant. All variables significant in univariate analysis were included in a multivariate backwards linear regression analysis $P \leq 0.05$.

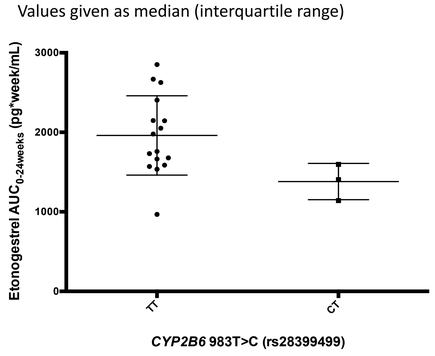
Characteristics	Efavirenz group (n=19)		
Age (years)	29 (23-35)		
Height (cm)	157 (150-165)		
Weight (kg)	56 (48-64)		
CD4 count (cells/mm^3)	449 (274-1072)		
Genotype frequencies			
CYP2B6 516G>T (rs3745274) (%)	GG	GT	TT
	32	58	11
CYP2B6 983T>C (rs28399499) (%)	TT	CT	CC
	84	16	0
CYP2B6 15582C>T (rs4803419) (%)	CC	CT	TT
	95	5	0
NR1I2 63396C>T (rs2472677) (%)	CC	CT	TT
	37	47	16
CYP3A4 293A>G (rs2740574) (%)	AA	AG	GG
	42	58	0
ABCB1 4036A>G (rs3842) (%)	AA	AG	GG
	58	21	21
ABCB1 3435C>T (rs1045642) (%)	CC	CT	TT
	68	32	0

Patient characteristics and genotype frequencies for each SNP.
Values given as median (interquartile range) or %

A)



B)



Difference in ENG AUC for each statistically significant SNP.
Values represented as mean (standard deviation)

***CYP2B6* 516G>T was associated with:**
- 43% lower log_{10} ENG C_{min} (P=0.003, β=-0.102) in TT compared to GG patients
- 34% lower log_{10} ENG $AUC_{0\text{-}24\ weeks}$ (P=0.008, β=-0.106) in TT compared to GG patients

***CYP2B6* 983T>C was associated with:**
- 37% lower log_{10} ENG C_{max} (P=0.003, β=-0.237) in CT compared to TT patients
- 20% lower log_{10} ENG $AUC_{0\text{-}24\ weeks}$ (P=0.016, β=-0.158) in CT compared to TT patients

EFV plasma concentration ($C_{12\text{-}14hrs}$) was:
- 76% higher in TT patients for *CYP2B6* 516G>T compared to GG patients
- 69% higher in CT patients for *CYP2B6* 983T>C compared to TT patients

RESULTS

log_{10} ENG C_{max}	Univariate linear regression			Multivariate linear regression		
	P value	β value (95% CI)	r^2	*P* value	β value (95% CI)	r^2
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			
***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}	Univariate linear regression			Multivariate linear regression		
	P value	β value (95% CI)	r^2	*P* value	β value (95% CI)	r^2
***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 $AUC_{0\text{-}24\ weeks}$	Univariate linear regression			Multivariate linear regression		
	P value	β value (95% CI)	r^2	*P* value	β value (95% CI)	r^2
***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**

Statistically significant associations between variables and ENG pharmacokinetic parameters.

	***CYP2B6* 516G>T (rs3745274)**			***CYP2B6* 983T>C (rs28399499)**		
	GG	GT	TT	TT	CT	CC
ENG C_{max} (pg/mL)	160 (158-185)	133 (102-207)	97 (85-109)	148 (109-207)	93 (75-102)	-
ENG C_{min} (pg/mL)	81 (63-84)	65 (57-76)	46 (40-52)	67 (53-81)	60 (57-62)	-
EFV $C_{12\text{-}14h}$ (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)	-

ENG and EFV pharmacokinetics for each statistically significant genotype.
Values given as median (interquartile range)

CONCLUSIONS

This study demonstrates a relationship between *CYP2B6* polymorphisms and alterations in ENG pharmacokinetics when ENG implants are combined with EFV.

We hypothesize that this relationship is mediated by an indirect effect of reduced EFV metabolism by CYP2B6, resulting in higher EFV concentrations. Supratherapeutic EFV concentrations may induce CYP3A4 to a greater extent, resulting in lower ENG exposure.

These results are consistent with previously observed associations between SNPs in *CYP2B6* and lower levonorgestrel implant pharmacokinetics when combined with EFV. (4)

Further study is required to confirm the mechanism of action of this association.

(1) Chappell *et al.* 2017. AIDS (2) Moser *et al.* 2012. Anal Bioanal Chem (3) Almond LM *et al.* 2005. J Antimicrob Chemother (4)Neary *et al.* 2017. Clin Pharmacol Ther