**Effect of patient genetics on etonogestrel pharmacokinetics when combined with efavirenz or nevirapine antiretroviral therapy**

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**Running title:** Patient genetics alter etonogestrel pharmacokinetics when co-administered with efavirenz or nevirapine.

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**Synopsis**

### Background

We previously demonstrated etonogestrel concentrations were 82% lower in women using etonogestrel contraceptive implants plus efavirenz-based ART, compared to 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 either 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 (600mg daily)-based ART and 19 receiving nevirapine (200mg twice daily)-based ART. Associations between patient genotype and etonogestrel pharmacokinetic parameters were determined through univariate and multivariate linear regression.

### Results

Within the control group, *CYP2B6* 983T>C was associated with 27% higher etonogestrel Cmax and 28% higher AUC0-24 weeks. In the efavirenz group *CYP2B6* 516G>T was associated with 43% lower etonogestrel Cmin and 34% lower AUC0-24 weeks. For participants receiving nevirapine, *NR1I2* 63396C>T was associated with 39% lower etonogestrel Cmin and 37% lower AUC0-24 weeks.

### 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 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 to 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 into study design and participants have been described previously by Chappell *et al*. 6 In brief, this pharmacogenetics sub-study included 57 of the 60 Ugandan women enrolled into the parent study, 19 receiving nevirapine (200 mg twice daily), 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 was not limited to, HIV-RNA >400 copies/mL in participants receiving ART, CD4+ cell count <350 cells/mm3 in the antiretroviral naïve group, and coadministration of medication contraindicated for use with etonogestrel or 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 minimise 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 post 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 post implant insertion. For nevirapine sampling, blood was drawn 11-13 hours after the participant’s last nevirapine dose. For efavirenz sampling was completed 12-14 hours after the last efavirenz dose. Etonogestrel concentrations were quantified from plasma through week 24 post etonogestrel implant placement, using HPLC/mass spectrometry. 24 For nevirapine and efavirenz quantification, HPLC was performed utilising validated methods. 25, 26 The pharmacokinetic parameters included in this study were AUC from entry to week 24 (AUC0-24 weeks), maximum concentration (Cmax), time to Cmax (Tmax) and minimum concentration (Cmin). Cmax and Cmin 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 manufacturers’ protocol (E.Z.N.A Blood DNA Mini Kit; Omega bio-tek; Norcross, GA). Genotyping was completed using real-time allelic discrimination PCR assay on a DNA Engine Chromo4 system (Bio-Rad Laboratories, Hercules, CA). The PCR protocol followed denaturation at 95°C for 10 minutes, followed by 50 cycles of amplification at 92°C for 15 seconds and annealing at 60°C for 1 minute 30 seconds. Samples were genotyped for the following SNPs utilising Taqman assays: *CYP2B6* 516G>T (rs3745274), 983T>C (rs28399499) and 15582C>T (rs4803419), *NR1I2* 63396C>T (rs2472677), *CYP3A4* 392G>A (rs2740574), *ABCB1* 4036A>G (rs3842) and 3435C>T (rs1045642) using Taqman Genotyping Master mix and corresponding Taqman Genotyping assays purchased from Thermo Fisher Scientific (Wilmington, DE). 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 median and IQR. The Shapiro-Wilk Test was used to test for normality, with a *P* ≤ 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* ≤ 0.2 for the univariate analysis were carried through to a linear backwards multivariate analysis with *P* ≤ 0.05 considered statistically significant. All statistical analyses were carried out using IBM SPSS Statistics v.24 (IBM Armonk, NY). All charts were produced using GraphPad Prism 6 (GraphPad Software, La Jolla, CA).

##### 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* 4036A>G, which compromises this SNPs interpretation. Statistically significant univariate and multivariate regression analysis results of each group are presented in Table 2. Full regression analysis results are shown in Supplementary Table 1.

#### Control group

Within the control group *CYP2B6* 983T>C was significantly associated with higher log10 etonogestrel Cmax (*P* = 0.013, β = 0.193) and higher log10 etonogestrel AUC0-24 weeks (*P* = 0.011, β = 0.188); equivalent to 10% higher etonogestrel Cmax and 76% higher etonogestrel AUC0-24weeks in participants heterozygous CT compared to those homozygous TT. *CYP3A4* 392G>A was also significantly associated with higher log10 etonogestrel Cmax (*P* = 0.028, β = 0.083) and higher log10 etonogestrel AUC0-24 weeks (*P* = 0.034, β = 0.076); equivalent to 64% higher etonogestrel Cmax and 63% higher etonogestrel AUC0-24weeks in participants homozygous G compared to those homozygous A (Table 2 and Table 3).

Efavirenz group

As shown in Table 2 and Figure 1, *CYP2B6* 516G>T was associated with a lower log10 etonogestrel Cmin (*P* = 0.003, β = -0.102) and lower log10 etonogestrel AUC0-24 weeks (*P* = 0.008, β = -0.106) for participants receiving efavirenz. This equates to 43% difference in etonogestrel Cmin and 34% difference in etonogestrel AUC0-24weeks between participants with homozygous G and homozygous T genotypes for *CYP2B6* 516G>T respectively (See Table 3).

*CYP2B6* 983T>C was associated with lower log10 etonogestrel Cmax (*P* = 0.003, β = -0.237) and lower log10 etonogestrel AUC0-24 weeks (*P* = 0.016, β = -0.158), which equates to a 37% difference in etonogestrel Cmax and a 20% difference in etonogestrel AUC0-24 weeks between participants who were homozygous T and heterozygous CT for *CYP2B6* 983T>C when prescribed efavirenz alongside the etonogestrel contraceptive implant (See Tables 2 and 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 week 4 visit (Table 4). Further, participants who were homozygous (TT) or heterozygous (GT) for CYP2B6 516G>T and those heterozygous CT for *CYP2B6* 983T>C had a median concentration below 90 pg/mL by the week 4 visit.

As anticipated, efavirenz plasma concentration (C12-14hrs) was 76% higher in participants homozygous T for *CYP2B6* 516G>T and 69% higher in participants heterozygous CT for *CYP2B6* 983T>C compared to participants who were homozygous T (Table 3).

Nevirapine group

For participants on nevirapine treatment, *NRI12* 63396C>T was associated with lower log10 etonogestrel Cmin (*P* = 0.010, β = -0.091) and lower log10 etonogestrel AUC0-24 weeks (*P* = < 0.001, β = -0.013); equivalent to 39% lower etonogestrel Cmin and 37% lower etonogestrel AUC0-24weeks in participants homozygous TT compared to those homozygous CC. *CYP2B6* 983T>C was associated with higher log10 etonogestrel Cmax (*P* = 0.013, β = 0.187), which equates to etonogestrel Cmax difference of 41% between homozygous T and heterozygous CT participants. *CYP3A4* 392G>A was associated with higher log10 etonogestrel AUC0-24 weeks (*P* = 0.004, β = 0.096), which equates to an 18% difference in log10 etonogestrel AUC0-24 weeks between homozygous G and homozygous A participants (Table 2 and Table 3).

Nevirapine median plasma concentration (C12-14hrs) was 7% lower in participants homozygous T for *NRI12* 63396C>T compared to participants homozygous C and 18% higher in participants heterozygous for *CYP2B6* 983T>C compared to participants homozygous T. Furthermore, for participants homozygous A for *CYP3A4* 392G>A nevirapine plasma concentration (C12-14hrs) was 10% higher than in participants homozygous G (Table 3).

**Discussion**

This study demonstrates associations between genetic variations in *CYP2B6* 516G>T and 983T>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 AUC0-24wks within homozygous T participants compared to homozygous G participants for *CYP2B6* 516G>T. For levonorgestrel AUC0-24wks, 64% lower results were observed for homozygous T participants compared to homozygous G for *CYP2B6* 516G>T. 7 Furthermore, 20% lower etonogestrel AUC0-24wks was seen between homozygous C versus heterozygous CT participants for *CYP2B6* 983T>C 7, similar to the 23% lower levonorgestrel AUC0-24wks observed between these genotypes within 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* 516G>T and 983T>C have been shown to result in reduced *CYP2B6* expression. 18, 29

In previous work, we reported an association between *NRI12* 63396C>T and higher levonorgestrel Tmax. Also, we observed an association between *CYP2B6* 516G>T and higher levonorgestrel Cmin and Cmax. 7 The consistent findings of these two studies strengthens 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 within women with variant alleles for *CYP2B6* SNPs who receive efavirenz and levonorgestrel- or etonogestrel-based contraceptive implants.

Within the nevirapine group, *NRI12* 63396C>T, *CYP3A4* 392G>A and *CYP2B6* 983T>C were associated with alterations in etonogestrel pharmacokinetics. The association of *CYP3A4* 392G>A with higher log10 etonogestrel AUC0-24weeks is a novel finding in this study. *CYP3A4* 392G>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 hypothesised 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 (Table 2 and 3), where *CYP3A4* 392G>A was associated with higher log10 etonogestrel AUC0-24weeks. The relationship between *CYP2B6* 983T>C and higher etonogestrel Cmax 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* 983T>C was associated with a 27% higher etonogestrel Cmax, between TT and CT genotype patients. Additionally these findings mirror that observed within our levonorgestrel study, where *CYP2B6* 516G>T was significantly associated with higher levonorgestrel Cmin and Cmax within the nevirapine group. 7 While these consistent findings support the legitimacy of an association, a biologic mechanism for this interaction is yet to be elucidated. The contradictory nature of the relationship between nevirapine pharmacokinetics and *CYP2B6* 983T>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 DDI 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 (PBPK) modelling to examine the effect of a reduction in efavirenz dose (600mg to 400mg) 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 (GM) at week 24 = 66 pg/mL: a 51% reduction in etonogestrel concentration from study week one) compared to that seen for levonorgestrel prescribed alongside efavirenz at study week 24 (GM at week 24 = 280 pg/mL: 31% reduction from in levonorgestrel concentration from study week one). 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 to 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* 4036A>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.

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### References

1. Fund TG. The Global Fund Results Report 2017 In: Wijnroks M, ed. <https://www.theglobalfund.org/media/6773/corporate_2017resultsreport_report_en.pdf>: The Global Fund, 2017; 50.

2. Organization WH. WHO statement on progestogen-only implants. World Health Organization, 2015.

3. Vieira CS, Bahamondes MV, de Souza RM et al. Effect of antiretroviral therapy including lopinavir/ritonavir or efavirenz on etonogestrel-releasing implant pharmacokinetics in HIV-positive women. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 2014; **66**: 378-85.

4. Leticee N, Viard J-P, Yamgnane A et al. Contraceptive failure of etonogestrel implant in patients treated with antiretrovirals including efavirenz. *Contraception* 2012; **85**: 425-7.

5. McCarty E, Keane H, Quinn K et al. Implanon® failure in an HIV-positive woman on antiretroviral therapy resulting in two ectopic pregnancies. *International journal of STD & AIDS* 2011; **22**: 413-4.

6. Chappell CA, Lamorde M, Nakalema S et al. Efavirenz decreases etonogestrel exposure: a pharmacokinetic evaluation of implantable contraception with antiretroviral therapy. *Aids* 2017; **31**: 1965-72.

7. Neary M, Lamorde M, Olagunju A et al. The effect of gene variants on levonorgestrel pharmacokinetics when combined with antiretroviral therapy containing efavirenz or nevirapine. *Clinical Pharmacology & Therapeutics* 2017; **102**: 529-36.

8. Maddox DD, Rahman Z. Etonogestrel (Implanon), another treatment option for contraception. *Pharmacy and Therapeutics* 2008; **33**: 337.

9. Moreno I, Quiñones L, Catalán J et al. Influence of CYP3A4/5 polymorphisms in the pharmacokinetics of levonorgestrel: a pilot study. *Biomédica* 2012; **32**: 570-7.

10. Istrate MA, Nussler AK, Eichelbaum M et al. Regulation of CYP3A4 by pregnane X receptor: The role of nuclear receptors competing for response element binding. *Biochemical and biophysical research communications* 2010; **393**: 688-93.

11. Swart M, Ren Y, Smith P et al. ABCB1 4036A> G and 1236C> T polymorphisms affect plasma efavirenz levels in South African HIV/AIDS patients. *Frontiers in genetics* 2012; **3**: 236.

12. Haas DW, Ribaudo HJ, Kim RB et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *Aids* 2004; **18**: 2391-400.

13. Rotger M, Colombo S, Furrer H et al. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. *Pharmacogenetics and genomics* 2005; **15**: 1-5.

14. Wyen C, Hendra H, Vogel M et al. Impact of CYP2B6 983T> C polymorphism on non-nucleoside reverse transcriptase inhibitor plasma concentrations in HIV-infected patients. *Journal of antimicrobial chemotherapy* 2008; **61**: 914-8.

15. Schipani A, Wyen C, Mahungu T et al. Integration of population pharmacokinetics and pharmacogenetics: an aid to optimal nevirapine dose selection in HIV-infected individuals. *Journal of antimicrobial chemotherapy* 2011: dkr087.

16. Hui K, Lee S, Lam T. Dose optimization of efavirenz based on individual CYP2B6 polymorphisms in Chinese patients positive for HIV. *CPT: pharmacometrics & systems pharmacology* 2016; **5**: 182-91.

17. Nightingale S, Chau TTH, Fisher M et al. Efavirenz and Metabolites in Cerebrospinal Fluid: Relationship with CYP2B6 c. 516G→ T Genotype and Perturbed Blood-Brain Barrier Due to Tuberculous Meningitis. *Antimicrobial agents and chemotherapy* 2016; **60**: 4511-8.

18. Solus JF, Arietta BJ, Harris JR et al. Genetic variation in eleven phase I drug metabolism genes in an ethnically diverse population. *Pharmacogenomics* 2004; **5**: 895-931.

19. Wang J, Sönnerborg A, Rane A et al. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. *Pharmacogenetics and genomics* 2006; **16**: 191-8.

20. Haas DW, Gebretsadik T, Mayo G et al. Associations between CYP2B6 polymorphisms and pharmacokinetics after a single dose of nevirapine or efavirenz in African Americans. *Journal of Infectious Diseases* 2009; **199**: 872-80.

21. Marzolini C, Rajoli R, Battegay M et al. Physiologically Based Pharmacokinetic Modeling to Predict Drug–Drug Interactions with Efavirenz Involving Simultaneous Inducing and Inhibitory Effects on Cytochromes. *Clinical pharmacokinetics* 2017; **56**: 409-20.

22. Mouly S, Lown KS, Kornhauser D et al. Hepatic but not intestinal CYP3A4 displays dose‐dependent induction by efavirenz in humans. *Clinical Pharmacology & Therapeutics* 2002; **72**: 1-9.

23. Hariparsad N, Nallani SC, Sane RS et al. Induction of CYP3A4 by efavirenz in primary human hepatocytes: comparison with rifampin and phenobarbital. *The Journal of Clinical Pharmacology* 2004; **44**: 1273-81.

24. Moser C, Zoderer D, Luef G et al. Simultaneous online SPE-LC-MS/MS quantification of six widely used synthetic progestins in human plasma. *Analytical and bioanalytical chemistry* 2012; **403**: 961-72.

25. Almond LM, Hoggard PG, Edirisinghe D et al. Intracellular and plasma pharmacokinetics of efavirenz in HIV-infected individuals. *Journal of antimicrobial Chemotherapy* 2005; **56**: 738-44.

26. Almond LM, Edirisinghe D, Dalton M et al. Intracellular and plasma pharmacokinetics of nevirapine in human immunodeficiency virus‐infected individuals. *Clinical Pharmacology & Therapeutics* 2005; **78**: 132-42.

27. Rodriguez S, Gaunt TR, Day IN. Hardy-Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *American journal of epidemiology* 2009; **169**: 505-14.

28. Diaz S, Pavez M, Moo-Young A et al. Clinical trial with 3-keto-desogestrel subdermal implants. *Contraception* 1991; **44**: 393-408.

29. Hofmann MH, Blievernicht JK, Klein K et al. Aberrant splicing caused by single nucleotide polymorphism c. 516G> T [Q172H], a marker of CYP2B6\* 6, is responsible for decreased expression and activity of CYP2B6 in liver. *Journal of Pharmacology and Experimental Therapeutics* 2008; **325**: 284-92.

30. Jin T, Yang H, Zhang J et al. Polymorphisms and phenotypic analysis of cytochrome P450 3A4 in the Uygur population in northwest China. *International journal of clinical and experimental pathology* 2015; **8**: 7083.

31. Riska P, Lamson M, MacGregor T et al. Disposition and biotransformation of the antiretroviral drug nevirapine in humans. *Drug Metabolism and Disposition* 1999; **27**: 895-901.

32. Neary M, Owen A. Pharmacogenetic considerations for HIV treatment in different ethnicities: an update. *Expert opinion on drug metabolism & toxicology* 2017; **13**: 1169-81.

33. Kimberly K. Scarsi LC, Shadia Nakalema, Kristin Darin, Ian Musinguzi, Isabella Kyohairwe, Pauline Byakika-Kibwika, Andrew Owen, Lee Winchester, Anthony Podany, Susan E. Cohn, David Back, Courtney V. Fletcher, Marco Siccardi, Mohammed Lamorde. Double-dose levonorgestrel does not fully overcome interaction with efavirenz. *Conference on Retroviruses and Opportunistic Infections*. Seattle Washington USA, 2019.

34. Chappell C. Pharmacologic Strategies for the Etonogestrel Implant in HIV-Infected Women.

35. Scarsi KK, Darin KM, Nakalema S et al. Unintended pregnancies observed with combined use of the levonorgestrel contraceptive implant and efavirenz-based antiretroviral therapy: a three-arm pharmacokinetic evaluation over 48 weeks. *Clinical Infectious Diseases* 2016; **62**: 675-82.

36. Roberts O, Rajoli RK, Back DJ et al. Physiologically based pharmacokinetic modelling prediction of the effects of dose adjustment in drug–drug interactions between levonorgestrel contraceptive implants and efavirenz-based ART. *Journal of Antimicrobial Chemotherapy* 2018; **73**: 1004-12.

Figure Legends

**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 (standard deviation) and compared by genotype for each of the single nucleotide polymorphisms significantly associated with etonogestrel AUC0-24weeks found through multivariate analysis (*P =* 0.05) within the efavirenz group (Graphs A and B) and the nevirapine group (Graphs C and D).

Supplementary materials

Table S1. Univariate and multivariate linear regression analysis for each study group.

ENG: etonogestrel. Univariate linear regression (*P =* ≤ 0.2) completed, all statistically significant results then carried through to multivariate linear regression analysis (*P =* ≤ 0.05). Statistically significant associations from the multivariate analysis are shown in bold type.

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| **Characteristics** | **Total (n = 57)** | | | **Control group (n = 19)** | | | **Efavirenz group (n = 19)** | | | **Nevirapine group (n = 19)** | | |
| 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/mm3) | 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 |

**Table 1. Characteristics of the study participants at entry.** Values shown as median (IQR) and percentage of population.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Efavirenz group | | | | | | |
| log10 ENG Cmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| *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 Tmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Weight (log10kg) | 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** |
| log10  ENG Cmin | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| ***CYP2B6* 516G>T(rs3745274)** | 0.003 | -0.102 (-0.2,0.0) | 0.423 | **0.003** | **-0.102 (-0.2,0.0)** | **0.423** |
| log10  ENG AUC0-24 weeks | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| ***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** | | | | | | |
| log10  ENG Cmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| ***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 |  |  |  |
| log10  ENG Cmin | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| *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** |
| log10  ENG AUC0-24 weeks | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| *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 | | | | | | |
| log10 ENG Cmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| ***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** |
| log10  ENG AUC0-24 weeks | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| ***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** |

Table 2. Statistically significant results from univariate and multivariate linear regression analysis within each study group. ENG: etonogestrel. Univariate linear regression (*P =* ≤0.2) completed, all statistically significant results then carried through to multivariate linear regression analysis (*P =* ≤0.05). All statistically significant variables from multivariate linear regression shown in bold type.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Allele frequency** | ***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** |
| 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 Cmax (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 Tmax (wk)** |  | | |  | | |  | | |  | | |
| 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 Cmin (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 AUC0-24weeks (pg\*wk/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 C12-14h (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 C11-13h (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) |

Table 3. Etonogestrel (ENG), Efavirenz (EFV) and Nevirapine (NVP) pharmacokinetic parameters shown as median (IQR), summarized by associated *CYP2B6, NR1I2* or *CYP3A4* genotype. EFV C12-14h (mg/L) and NVP C11-13h (mg/L) determined from individual participant’s geometric mean value calculated from concentration measured at study entry, week 1, 4, 12, 24 and 48 summarized for the group as median (IQR).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *CYP2B6* 516G>T (rs3745274) | **ENG concentration (pg/mL)** | | | |
| **Week 1** | **Week 4** | **Week 12** | **Week 24** |
| 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) | **Week 1** | **Week 4** | **Week 12** | **Week 24** |
| 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) | - | - | - | - |

**Table 4.** **Etonogestrel (ENG) concentration per week of study summarised by significant CYP2B6 SNP genotype within the efavirenz group.** Values shown 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 is represented by mean (standard deviation) and compared by genotype for each of the single nucleotide polymorphisms significantly associated with etonogestrel AUC0-24weeks found through multivariate analysis (*P =* 0.05) within the efavirenz group (Graphs A and B) and the nevirapine group (Graphs C and D).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Efavirenz Group** | | | | | | |
| log10 ENG Cmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.323 | 0.393 (-0.4,1.2) | 0.058 |  |  |  |
| log10 Weight (log10kg) | 0.324 | 0.508 (-0.5,1.6) | 0.057 |  |  |  |
| log10 Height (log10kg) | 0.627 | 1.090 (-3.6,5.7) | 0.014 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.794 | 0.034 (-0.2,0.3) | 0.004 |  |  |  |
| *CYP2B6* 516G>T(rs3745274) | 0.135 | -0.085 (-0.2,0.0) | 0.126 | 0.054 | -0.084 (-0.2,0.0) | 0.518 |
| ***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 | 0.260 | -0.166 (-0.5,0.1) | 0.618 |
| *CYP3A4* 392G>A (rs2740574) | 0.691 | 0.029 (-0.1,0.2) | 0.010 |  |  |  |
| *NR1I2* 63396C>T (rs2472677) | 0.481 | 0.036 (-0.1,0.1) | 0.030 |  |  |  |
| *ABCB1* 4036A>G (rs3842) | 0.110 | 0.068 (0.0,0.2) | 0.144 | 0.067 | 0.060 (0.0,0.1) | 0.581 |
| *ABCB1* 3435C>T (rs1045642) | 0.970 | 0.003 (-0.2,0.2) | 0.000 |  |  |  |
| ENG Tmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.590 | -0.993 (-4.8,2.8) | 0.017 |  |  |  |
| log10 Weight (log10kg) | 0.199 | 3.005 (-1.7,7.8) | 0.095 | 0.550 | 1.420 (-3.5,6.3) | 0.234 |
| log10 Height (log10kg) | 0.595 | 5.458 (-15.8,26.7) | 0.017 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.556 | -0.355 (-1.6,0.9) | 0.021 |  |  |  |
| ***CYP2B6* 516G>T(rs3745274)** | 0.045 | 0.507 (0.0,1.0) | 0.216 | **0.045** | **0.507 (0.0,1.0)** | **0.216** |
| *CYP2B6* 983T>C (rs28399499) | 0.678 | -0.188 (-1.1,0.7) | 0.010 |  |  |  |
| *CYP2B6* 15582C>T (rs4803419) | 0.821 | -0.167 (1.7,1.4) | 0.003 |  |  |  |
| *CYP3A4* 392G>A (rs2740574) | 0.252 | 0.375 (-0.3,1,0) | 0.076 |  |  |  |
| *NR1I2* 63396C>T (rs2472677) | 0.267 | -0.259 (-0.7,0.2) | 0.072 |  |  |  |
| *ABCB1* 4036A>G (rs3842) | 0.662 | 0.089 (-0.3,0.5) | 0.012 |  |  |  |
| *ABCB1* 3435C>T (rs1045642) | 0.513 | -0.231 (-1.0,0.5) | 0.026 |  |  |  |
| log10  ENG Cmin | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.868 | -0.044 (-0.6,0.5) | 0.002 |  |  |  |
| log10 Weight (log10kg) | 0.745 | -0.112 (-0.8,0.6) | 0.006 |  |  |  |
| log10 Height (log10kg) | 0.619 | -0.737 (-3.8,2.3) | 0.015 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.953 | 0.005 (-0.2,0.2) | 0.000 |  |  |  |
| ***CYP2B6* 516G>T(rs3745274)** | 0.003 | -0.102 (-0.2,0.0) | 0.423 | **0.003** | **-0.102 (-0.2,0.0)** | **0.423** |
| *CYP2B6* 983T>C (rs28399499) | 0.395 | -0.055 (-0.2,0.1) | 0.043 |  |  |  |
| *CYP2B6* 15582C>T (rs4803419) | 0.665 | -0.046 (-0.3,0.2) | 0.011 |  |  |  |
| *CYP3A4* 392G>A (rs2740574) | 0.802 | 0.012 (-0.1,0.1) | 0.004 |  |  |  |
| *NR1I2* 63396C>T (rs2472677) | 0.474 | 0.024 (0.0,0.1) | 0.031 |  |  |  |
| *ABCB1* 4036A>G (rs3842) | 0.572 | 0.016 (0.0,0.1) | 0.019 |  |  |  |
| *ABCB1* 3435C>T (rs1045642) | 0.664 | 0.022 (-0.1,0.1) | 0.011 |  |  |  |
| log10  ENG AUC0-24 weeks | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.342 | 0.308 (-0.4,1.0) | 0.053 |  |  |  |
| log10 Weight (log10kg) | 0.859 | -0.076 (-1.0,0.8) | 0.002 |  |  |  |
| log10 Height (log10kg) | 0.316 | -1.807 (-5.5,1.9) | 0.059 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.773 | -0.031 (-0.3,0.2) | 0.005 |  |  |  |
| ***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** |
| *CYP2B6* 15582C>T (rs4803419) | 0.671 | -0.056 (-0.3,0.2) | 0.011 |  |  |  |
| *CYP3A4* 392G>A (rs2740574) | 0.403 | 0.049 (-0.1,0.2) | 0.041 |  |  |  |
| *NR1I2* 63396C>T (rs2472677) | 0.694 | 0.017 (-0.1,0.1) | 0.009 |  |  |  |
| *ABCB1* 4036A>G (rs3842) | 0.259 | 0.040 (0.0,0.1) | 0.074 |  |  |  |
| *ABCB1* 3435C>T (rs1045642) | 0.640 | 0.029 (-0.1,0.2) | 0.013 |  |  |  |
| **Nevirapine Group** | | | | | | |
| log10  ENG Cmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.704 | 0.186 (-0.8,1.2) | 0.009 |  |  |  |
| log10 Weight (log10kg) | 0.835 | -0.075 (-0.8,0.7) | 0.003 |  |  |  |
| log10 Height (log10kg) | 0.655 | -0.779 (-4.4,2.8) | 0.012 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.343 | 0.166 (-0.2,0.5) | 0.053 |  |  |  |
| *CYP2B6* 516G>T(rs3745274) | 0.443 | -0.047 (-0.2,0.1) | 0.035 |  |  |  |
| ***CYP2B6* 983T>C (rs28399499)** | 0.013 | 0.187 (0.0,0.3) | 0.313 | **0.013** | **0.187 (0.0,0.3)** | **0.313** |
| *CYP2B6* 15582C>T (rs4803419) | 0.505 | -0.061 (-0.3,0.1) | 0.027 |  |  |  |
| *CYP3A4* 392G>A (rs2740574) | 0.286 | -0.052 (-0.2,0.5) | 0.067 |  |  |  |
| *NR1I2* 63396C>T (rs2472677) | 0.058 | -0.091 (-0.2,0.0) | 0.196 | 0.352 | -0.045 (-0.1,0.1) | 0.351 |
| *ABCB1* 4036A>G (rs3842) | 0.742 | -0.013 (-0.1,0.1) | 0.007 |  |  |  |
| *ABCB1* 3435C>T (rs1045642) | 0.992 | -0.001 (-0.2,0.2) | 0.000 |  |  |  |
| ENG Tmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| Age (years) |  |  |  |  |  |  |
| log10 weight (log10kg) |  |  |  |  |  |  |
| log10 Height (log10kg) |  |  |  |  |  |  |
| log10 CD4 (log10cells/mm3) |  |  |  |  |  |  |
| *CYP2B6* 516G>T(rs3745274) |  |  |  |  |  |  |
| *CYP2B6* 983T>C (rs28399499) |  |  |  |  |  |  |
| *CYP2B6* 15582C>T (rs4803419) |  |  |  |  |  |  |
| *CYP3A4* 392G>A (rs2740574) |  |  |  |  |  |  |
| *NR1I2* 63396C>T (rs2472677) |  |  |  |  |  |  |
| *ABCB1* 4036A>G (rs3842) |  |  |  |  |  |  |
| *ABCB1* 3435C>T (rs1045642) |  |  |  |  |  |  |
| log10  ENG Cmin | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.414 | 0.309 (-0.5,1.1) | 0.040 |  |  |  |
| log10 Weight (log10kg) | 0.927 | -0.026 (-0.6,0.6) | 0.001 |  |  |  |
| log10 Height (log10kg) | 0.659 | -0.599 (3.4,2.2) | 0.012 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.492 | 0.094 (-0.2,0.3) | 0.028 |  |  |  |
| *CYP2B6* 516G>T(rs3745274) | 0.972 | 0.002 (-0.1,0.1) | 0.000 |  |  |  |
| *CYP2B6* 983T>C (rs28399499) | 0.062 | 0.114 (0.0,0.2) | 0.190 | 0.387 | 0.053 (-0.1,0.2) | 0.028 |
| *CYP2B6* 15582C>T (rs4803419) | 0.417 | -0.058 (-0.2,0.1) | 0.039 |  |  |  |
| *CYP3A4* 392G>A (rs2740574) | 0.691 | -0.015 (-0.1,0.1) | 0.009 |  |  |  |
| ***NR1I2* 63396C>T (rs2472677)** | 0.010 | -0.091 (-0.2,0.0) | 0.329 | **0.010** | **-0.091 (-0.2,0.0)** | **0.028** |
| *ABCB1* 4036A>G (rs3842) | 0.886 | 0.005 (-0.1,0.1) | 0.001 |  |  |  |
| *ABCB1* 3435C>T (rs1045642) | 0.333 | 0.068 (-0.1,0.2) | 0.055 |  |  |  |
| log10  ENG AUC0-24 weeks | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.880 | -0.067 (-1.0,0.9) | 0.001 |  |  |  |
| log10 Weight (log10kg) | 0.833 | -0.069 (-0.7,0.6) | 0.003 |  |  |  |
| log10 Height (log10kg) | 0.674 | -0.666 (-4.0,2.6) | 0.011 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.346 | 0.150 (-0.2,0.5) | 0.052 |  |  |  |
| *CYP2B6* 516G>T(rs3745274) | 0.836 | -0.012 (-0.1,0.1) | 0.003 |  |  |  |
| *CYP2B6* 983T>C (rs28399499) | 0.080 | 0.125 (0.0,0.3) | 0.170 | 0.177 | 0.072 (0.0,0.2) | 0.685 |
| *CYP2B6* 15582C>T (rs4803419) | 0.465 | -0.061 (-0.2,0.1) | 0.032 |  |  |  |
| ***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 | **<0.000** | **-0.139 (-0.2,-0.1)** | **0.643** |
| *ABCB1* 4036A>G (rs3842) | 0.859 | -0.007 (-0.1,0.1) | 0.002 |  |  |  |
| *ABCB1* 3435C>T (rs1045642) | 0.730 | 0.029 (-0.1,0.2) | 0.007 |  |  |  |
| **Control Group** | | | | | | |
| log10 ENG Cmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.907 | 0.061 (-1.0,1.1) | 0.001 |  |  |  |
| log10 Weight (log10kg) | 0.594 | -0.186 (-0.9,0.5) | 0.017 |  |  |  |
| log10 Height (log10kg) | 0.823 | -0.434 (-4.5,3.6) | 0.003 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.839 | 0.042 (-0.4,0.5) | 0.003 |  |  |  |
| *CYP2B6* 516G>T(rs3745274) | 0.652 | -0.024 (-0.1,0.1) | 0.012 |  |  |  |
| ***CYP2B6* 983T>C (rs28399499)** | 0.053 | 0.159 (0.0,0.3) | 0.203 | **0.013** | **0.193 (0.0,0.3)** | **0.416** |
| *CYP2B6* 15582C>T (rs4803419) | 0.896 | -0.014 (-0.2,0.2) | 0.001 |  |  |  |
| ***CYP3A4* 392G>A (rs2740574)** | 0.133 | 0.063 (0.0,0.1) | 0.128 | **0.028** | **0.083 (0.0,0.2)** | **0.416** |
| *NR1I2* 63396C>T (rs2472677) | 0.223 | -0.051 (-0.1,0.0) | 0.086 |  |  |  |
| *ABCB1* 4036A>G (rs3842) | 0.784 | 0.017 (-0.1,0.1) | 0.005 |  |  |  |
| *ABCB1* 3435C>T (rs1045642) | 0.570 | 0.039 (-0.1,0.2) | 0.019 |  |  |  |
| ENG Tmax | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) |  |  |  |  |  |  |
| log10 Weight (log10kg) |  |  |  |  |  |  |
| log10 Height (log10kg) |  |  |  |  |  |  |
| log10 CD4 (log10cells/mm3) |  |  |  |  |  |  |
| *CYP2B6* 516G>T(rs3745274) |  |  |  |  |  |  |
| *CYP2B6* 983T>C (rs28399499) |  |  |  |  |  |  |
| *CYP2B6* 15582C>T (rs4803419) |  |  |  |  |  |  |
| *CYP3A4* 392G>A (rs2740574) |  |  |  |  |  |  |
| *NR1I2* 63396C>T (rs2472677) |  |  |  |  |  |  |
| *ABCB1* 4036A>G (rs3842) |  |  |  |  |  |  |
| *ABCB1* 3435C>T (rs1045642) |  |  |  |  |  |  |
| log10  ENG Cmin | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.433 | -0.459 (-1.7,0.7) | 0.037 |  |  |  |
| log10 Weight (log10kg) | 0.278 | -0.422 (-1.2,0.4) | 0.069 |  |  |  |
| log10 Height (log10kg) | 0.737 | 0.736 (-3.8,5.3) | 0.007 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.828 | 0.050 (-0.4,0.5) | 0.003 |  |  |  |
| *CYP2B6* 516G>T(rs3745274) | 0.894 | 0.008 (-0.1,0.1) | 0.001 |  |  |  |
| *CYP2B6* 983T>C (rs28399499) | 0.130 | 0.144 (0.0,0.3) | 0.129 |  |  |  |
| *CYP2B6* 15582C>T (rs4803419) | 0.958 | 0.006 (-0.2,0.3) | 0.000 |  |  |  |
| *CYP3A4* 392G>A (rs2740574) | 0.494 | 0.033 (0.1,0.1) | 0.028 |  |  |  |
| *NR1I2* 63396C>T (rs2472677) | 0.897 | -0.006 (-0.1,0.1) | 0.001 |  |  |  |
| *ABCB1* 4036A>G (rs3842) | 0.767 | 0.020 (-0.1,0.2) | 0.005 |  |  |  |
| *ABCB1* 3435C>T (rs1045642) | 0.863 | -0.013 (-0.2,0.1) | 0.002 |  |  |  |
| log10  ENG AUC0-24 weeks | Univariate linear regression | | | Multivariate linear regression | | |
| *P* value | β value (95% CI) | r2 | *P* value | β value (95% CI) | r2 |
| log10 Age ( log10years) | 0.342 | -0.464 (-1.4,0.5) | 0.053 |  |  |  |
| log10 Weight (log10kg) | 0.475 | -0.235 (-0.9,0.4) | 0.030 |  |  |  |
| log10 Height (log10kg) | 0.971 | -0.067 (-3.9,3.8) | 0.000 |  |  |  |
| log10 CD4 (log10cells/mm3) | 0.804 | 0.048 (-0.4,0.5) | 0.004 |  |  |  |
| *CYP2B6* 516G>T(rs3745274) | 0.807 | -0.012 (-0.1,0.1) | 0.004 |  |  |  |
| ***CYP2B6* 983T>C (rs28399499)** | 0.043 | 0.156 (0.0,0.3) | 0.219 | **0.011** | **0.188 (0.0,0.3)** | **0.415** |
| *CYP2B6* 15582C>T (rs4803419) | 0.939 | -0.008 (-0.2,0.2) | 0.000 |  |  |  |
| ***CYP3A4* 392G>A (rs2740574)** | 0.160 | 0.056 (0.0,0.1) | 0.113 | **0.034** | **0.076 (0.0,0.1)** | **0.415** |
| *NR1I2* 63396C>T (rs2472677) | 0.366 | -0.036 (-0.1,0.0) | 0.048 |  |  |  |
| *ABCB1* 4036A>G (rs3842) | 0.770 | 0.017 (-0.1,0.1) | 0.005 |  |  |  |
| *ABCB1* 3435C>T (rs1045642) | 0.709 | 0.024 (-0.1,0.2) | 0.008 |  |  |  |

Supplementary table 1. Univariate and multivariate linear regression analysis for each study group. ENG: etonogestrel. Univariate linear regression (*P =* ≤ 0.2) completed, all statistically significant results then carried through to multivariate linear regression analysis (*P =* ≤ 0.05). Statistically significant associations from the multivariate analysis are shown in bold type.