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Title: Influence of *UGT1A1* and *SLCO1B1* Polymorphisms and Efavirenz on Bilirubin
Disposition in Healthy Volunteers

Authors: Kimberly S. Collins PhD, Ingrid F. Metzger, PhD, Brandon T. Gufford, PharmD, PhD,
Jessica Lu, MS, Elizabeth B. Medeiros, MS, Victoria M. Pratt, PhD, Todd C. Skaar, PhD,
Zeruesenay Desta, PhD

Affiliations: Department of Medicine: Division of Clinical Pharmacology (KSC, IFM, BTG, JL,
TCS, ZD), Department of Medical and Molecular Genetics (EBM, VMP), Indiana University
School of Medicine, Indianapolis, IN

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Running Title: Induction of bilirubin disposition by efavirenz

Corresponding Author: Zeruesenay Desta, PhD

Address: Indiana University School of Medicine, Division of Clinical Pharmacology, 950 W

Walnut St, R2 building, Rm E425, Indianapolis, IN 46202

Email: zdesta@iu.edu

Telephone: +1 317 274 2823

Fax: +1 317 274 2704

Number of Text Pages: 16

Number of Tables: 3

Number of Figures: 4

Number of References: 40

Number of Words in Abstract: 217

Number of Words in Introduction: 749

Number of Words in Discussion: 1277

Abbreviations: CPIC: Clinical Pharmacogenetics Implementation Consortium, HAART: highly active antiretroviral therapy, LD: linkage disequilibrium, MRP2: multidrug resistance-associated protein 2, OATP1B1: organic anion transporting polypeptide 1B1, PCR: polymerase chain reactions, SNP: single nucleotide polymorphism, UGT1A1: uridine diphosphate glucuronosyltransferase family 1 member A1

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Abstract:

Chronic administration of efavirenz is associated with decreased serum bilirubin levels, probably through induction of *UGT1A1*. We assessed the impact of efavirenz monotherapy and UGT1A1 phenotypes on total, conjugated, and unconjugated serum bilirubin levels in healthy volunteers. Healthy volunteers were enrolled into a clinical study designed to address efavirenz pharmacokinetics, drug interactions, and pharmacogenetics. Volunteers received multiple oral doses (600 mg/d for 17 days) of efavirenz. Serum bilirubin levels were obtained at study entry and 1 week after completion of the study. DNA genotyping was performed for *UGT1A1* (*80 (C>T), *6 (G>A), *28 (TA₇), *36 (TA₅) and *37 (TA₈)) and for *SLCO1B1* (*5 (521T>C) and *1b (388A>G) variants. Diplotype predicted phenotypes were classified as normal, intermediate and slow metabolizers. Compared to bilirubin levels at screening, treatment with efavirenz significantly reduced total, conjugated, and unconjugated bilirubin. After stratification by UGT1A1 phenotypes, there was a significant decrease in total bilirubin among all phenotypes, conjugated bilirubin among intermediate metabolizer, and unconjugated bilirubin among normal and intermediate metabolizers. The data also show that *UGT1A1* genotype predicts serum bilirubin levels at baseline, but this relationship is lost after efavirenz treatment. *SLCO1B1* genotypes did not predict bilirubin levels at baseline or after efavirenz treatment. Our data suggest that efavirenz may alter bilirubin disposition mainly through induction of UGT1A1 metabolism and efflux through MRP2.

Significance Statement:

Efavirenz likely alters the pharmacokinetics of coadministered drugs, potentially causing lack of efficacy or increased adverse effects, as well as the disposition of endogenous compounds

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relevant in homeostasis through upregulation of UGT1A1 and MRP2. Measurement of unconjugated and conjugated bilirubin during new drug development may provide mechanistic understanding regarding enzyme and transporters modulated by the new drug.

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Introduction:

The human immunodeficiency virus (HIV) type 1 infection, and the acquired immuno-deficiency syndrome (AIDS) associated with it, remains a major public health problem, particularly in low-income countries. The introduction of highly active antiretroviral therapy (HAART) in 1996 was a landmark treatment change that considerably improved the prognosis of patients infected with HIV by substantially reducing morbidity and mortality from HIV/AIDs and by reducing incidence of HIV infection (Palella et al., 1998). Until recently, HAART regimens containing the nonnucleoside HIV-1 reverse transcriptase inhibitor efavirenz have dominated the HIV/AIDs therapy as the preferred first-line in treatment naïve patients world-wide (Staszewski et al., 1999; Gulick et al., 2004). Efavirenz-based regimens have moved to second line drugs in 2015 in the developed world due to the development of safe and effective antiretroviral drugs. However, efavirenz-based therapy is still widely used in many low-income countries with HIV epidemic. In addition, many patients who have been on stable efavirenz-based therapy continue this treatment (Vitoria et al., 2018).

Efavirenz shows multiple interactions with drug metabolizing enzymes and possibly transporters (Sanchez-Martin et al., 2016; Molto et al., 2017). As a result, efavirenz alters the pharmacokinetics of numerous drugs. These interactions can lead to reduced/lack of efficacy or increased adverse reactions. Previously, we have shown that efavirenz monotherapy affects total bilirubin disposition in healthy volunteers (Metzger et al., 2014). This effect was highly variable among subjects. The processes involved in the hepatic disposition of bilirubin are depicted in Figure 1. Unconjugated bilirubin enters into hepatocytes from blood in part via sinusoidal

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membrane-bound organic anion transporting polypeptides OATP1B1 and OATP1B3 (OATP1B1/3) (Keppler et al., 2014) and subsequently undergo conjugation with glucuronic acid by (UDP)-glucuronosyltransferase 1A1 (UGT1A1) to form bilirubin mono- and di-glucuronides (Crawford et al., 1992). The glucuronides formed are either secreted into the bile mainly by multi-drug resistance-associated protein 2 (*MRP2*), located at the canalicular membrane of hepatocytes (Jedlitschky et al., 1997) or efflux transported into the blood by *MRP3* located in the hepatocyte sinusoidal membrane and from the blood they are reuptaken back to hepatocytes via OATP1B1/3 (van de Steeg et al., 2012; Keppler 2014). In humans, impaired bilirubin conjugation or transport leads to various degree of hyperbilirubinemia and are observed in several inherited disorders such as Crigler-Najjar syndrome type I and type II and Gilbert syndrome (impaired or absence of bilirubin conjugation resulting in unconjugated hyperbilirubinemia) (Bosma et al., 1995; Kadakol et al., 2000), Rotor-syndrome (absence of OATP1B1/3 characterized by Predominantly conjugated (and to a lesser extent unconjugated) hyperbilirubinemia due to deficient uptake transport of bilirubin by is seen in (van de Steeg et al., 2012), while conjugated hyperbilirubinemia due to deficient *MRP2*-mediated efflux transport of bilirubin glucuronides is seen in Dublin-Johnson syndrome (Sticova and Jirsa, 2013).

Because the *UGT1A1* gene is a highly polymorphic enzyme, there is wide variability in enzyme activity that is associated with drug response and toxicity as well as altered bilirubin conjugation (Ah et al., 2008; Chiddarwar et al., 2017). The *UGT1A1**28 (TA₇), a dinucleotide repeat polymorphism in the TATA sequence of the promoter region of *UGT1A1*, is the most common allele associated with reduced enzyme expression and activity as well as increased serum unconjugated hyperbilirubinemia. The *UGT1A1**28 and other *UGT1A1* missense variants have

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been implicated in Gilbert syndrome, an autosomal recessive unconjugated hyperbilirubinemia (Bosma et al., 1995). The Clinical Pharmacogenetics Implementation Consortium (CPIC) has published guidelines providing recommendations for the use of atazanavir, a protease inhibitor to treat HIV-1, based on *UGT1A1* genotypes (Gammal et al., 2016). This guideline indicate that individuals that carry two decreased function *UGT1A1* alleles are at high risk for jaundice and non-adherence and recommend physicians to consider an alternative drug. However, *UGT1A1**28 is a TA repeat and routine genotyping using conventional methods is challenging; however, *UGT1A1**80 (c.-364C>T) has been shown to be in high linkage disequilibrium (LD) with *UGT1A1**28 (TA₇) and *37 (TA₈) and may serve as a tag-SNP (single nucleotide polymorphism) (Gammal et al., 2016).

In this study, we hypothesize that efavirenz reduces both unconjugated and conjugated bilirubin levels primarily through induction of *UGT1A1* and/or *MRP2*, respectively. We also explored: 1) whether variants in the *UGT1A1* and *SLCO1B1* genes influences efavirenz induction of *UGT1A1* and OATP1B1/3; and 2) *UGT1A1**80 could serve as easy to use surrogate for *UGT1A1**28 genotyping. To address these objectives, baseline (at study entry) plasma total, conjugated and unconjugated bilirubin levels were compared with those obtained 1 week after a 17-day oral treatment with 600 mg/day efavirenz in healthy volunteers genotyped for variants in the *UGT1A1* and *SLCO1B1* genes.

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Methods

Study Population

Eligible healthy nonsmoking 18 to 49 years old male and female volunteers (N = 136) who were within 32% of their ideal body weight and were not taking any prescription and nonprescription medications were enrolled into two clinical trials that were designed to address efavirenz metabolism, pharmacokinetics, pharmacogenetics and drug interactions. Study subjects were judged healthy by medical history and standard screening laboratory tests. Inclusion and exclusion criteria of the subjects have been described in detail in our previous publications (Metzger et al., 2014; Robarge et al., 2016; Masters et al., 2016). Blood samples were obtained for DNA genotyping. The studies were approved by the Indiana University School of Medicine Institutional Review Board and each subject signed an informed consent and HIPAA documents before enrollment. The trials were conducted at the Clinical Research Center of the Indiana Clinical and Translational Sciences Institute and were registered at <http://www.clinicaltrials.gov> (trial identifiers NCT00668395 and NCT02401256).

Study design

The two protocols (NCT00668395 and NCT02401256) were primarily designed to test metabolism, pharmacokinetics, pharmacogenetics and drug interactions of efavirenz (single 600-mg oral dose) and after multiple doses (after 600 mg/d for 17 days orally). The study designs of these trials have been described in detail elsewhere (Metzger et al., 2014; Robarge et al., 2016; Masters et al., 2016). Among others, standard laboratory results including bilirubin (total,

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conjugated and unconjugated) were obtained during the screening phase (i.e., baseline, before any drug administration) and at exit (i.e., approximately 1 week after completion of 600 mg/day oral dose of efavirenz for 17 days). In this report, we determined the influence of efavirenz chronic administration on bilirubin disposition in these trials by comparing exit plasma bilirubin concentrations (total, unconjugated and conjugated) with those values obtained at baseline (screening).

***UGT1A1* and *SLCO1B1* Genotyping**

Genomic DNA extracted from human blood (n=136) was used for genotyping. DNA genotyping for *UGT1A1**80 (rs887829; -364C>T), *UGT1A1**6 (rs4148323; 211G>A, G71R), *SLCO1B1**5 (rs4149056; 521T>C, V174A), and *SLCO1B1**1b (rs2306283; 388A>G, N130D) was performed using TaqMan™ genotyping assays (ThermoFisher, Waltham, MA) according to manufacturer's protocol on the QuantStudio 12K Flex or ViiA 7 Real-Time PCR Systems (ThermoFisher Scientific, Waltham, MA). Genotyping for *UGT1A1**28 (TA₇), *36(TA₅), *37(TA₈) (rs8175347) was performed by polymerase chain reaction (PCR) amplification and capillary electrophoresis using a protocol adapted from Huang CK et al (Huang et al., 2007). Briefly, 12 uL CloneAmp HiFi PCR Premix (Takara Bio USA, Inc., Mountain View, CA), 10.8 uL water, 0.6 uL forward primer 5' (6-Fam) CGTGACACAGTCAAACATTAAGTT (0.24 μM:final concentration), 0.6 μL Reverse Primer 5' CAGCAGTGGCTGCCATCCAC (0.24 μM:final concentration) was PCR amplified with an initial denaturation at 98°C for 5 minutes, followed by 40 cycles of 98°C for 10s, 64°C for 5s, and 72°C for 5s, and a final extension at 72°C for 7 min. Capillary electrophoresis was performed on an Applied Biosystems 3130xL (ThermoFisher Scientific,

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Waltham MA). CPIC guidelines were used to identify UGT1A1 and SLCO1B1 phenotypes based on genotypes (Wilke et al., 2012; Gammal et al., 2016). Other variants that were genotyped in this population were described elsewhere (Robarge et al., 2017; Burgess et al., 2018).

Data Analysis

Total, conjugated, and unconjugated at screening versus exit bilirubin levels were compared using a Wilcoxon matched-pairs signed rank test for non-stratified and stratified UGT1A1 phenotypic groups. Total, conjugated, and unconjugated bilirubin level changes were compared across predicted UGT1A1 phenotypes using Kruskal-Wallis followed by Dunn's post hoc test. A p -value <0.05 was considered statistically significant. All analyses were performed using Graphpad Prism 5 (La Jolla, CA). Haplotype analysis and linkage disequilibrium was calculated using SHEsis (Shi and He, 2005; Li et al., 2009).

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Results

Demographics

Demographic characteristics of the study participants are listed in Table 1. The study included 136 volunteers, mostly male (59%) and self-identified white (65%). Both screening and exit total bilirubin concentrations were available for analysis in 133 study participants, whereas conjugated and unconjugated bilirubin levels were obtained in 65 and 67 subjects, respectively. The remaining subjects were missing either a screening or exit bilirubin measurement and are excluded from paired analysis only.

UGT1A1 and *SLCO1B1* genotypes and genotype-predicted phenotype frequencies

There were 136 volunteers genotyped for *UGT1A1* rs887829, rs8175347, rs4148323 and *SLCO1B1* rs4149056 and rs2306283 (Table 2). One goal of this study was to test whether *UGT1A1**80 could be used as a tag-SNP for *UGT1A1**28. *UGT1A1**80 was observed at a minor allele frequency of 32.4%, *UGT1A1**28 and *UGT1A1**37 were observed at a frequency of 33.5%, and *UGT1A1**6 at a frequency of 0.37%. The *UGT1A1**80 is in high linkage disequilibrium in our study population with *UGT1A1**28 and *UGT1A1**37 ($r^2=0.96$); however, this was not perfect and 2% of subjects would be misclassified as *UGT1A1**28 when *80 is used as a tag-SNP. Genotype-predicted phenotypes for *UGT1A1* in the healthy volunteers were as follows: 44%, 47%, and 9%, genotypic normal, intermediate, and slow metabolizers, respectively (Table 3). *SLCO1B1**5 and *SLCO1B1**1b genotype count and frequencies are described in Table 2 and genotype-predicted phenotypes in Table 3.

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Efavirenz treatment leads to reduced bilirubin levels

Compared to screening values, exit bilirubin levels were significantly decreased after efavirenz treatment for total bilirubin (33%, $P<0.0001$), conjugated bilirubin (31%, $P=0.0004$) and unconjugated bilirubin (36%, $P<0.0001$) (Figure 2A, B and C, respectively).

UGT1A1 polymorphisms are associated with inter-individual variability in total and unconjugated bilirubin levels

Total bilirubin levels at the start of the study (screening) vary depending on UGT1A1 metabolizer status. There was 81% and 63% higher total bilirubin levels in slow metabolizers, respectively, compared to normal ($P<0.01$) and intermediate ($P<0.05$) metabolizers (Figure 3A). However, this genotype-dependent effect is lost after chronic efavirenz dosing (exit), although the slow metabolizers have 50% and 46% higher total bilirubin levels on average compared to the normal and intermediate metabolizer status, respectively (Figure 3B). No statistically significant changes were observed in conjugated bilirubin levels across UGT1A1 phenotypes at screening and exit (Figure 3C and D). Unconjugated bilirubin levels were 111% ($P<0.001$) and 73% ($P<0.010$) higher in the slow metabolizer group compared to the normal and intermediate metabolizers, respectively (Figure 3E). These differences were not statistically significant after chronic efavirenz treatment (Figure 3F).

The effect of efavirenz on total bilirubin levels stratified by each UGT1A1 genotypes is illustrated in Figure 4A. Total bilirubin levels significantly decreased after completion of efavirenz treatment in all genotypic group: normal (29%, $P<0.0001$), intermediate (35%, $P<0.0001$), and slow (41%, $P=0.0078$) metabolizers (Figure 4A). Conjugated bilirubin levels

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were significantly reduced only among intermediate metabolizers (44%; $P=0.004$) (Figure 4B) and unconjugated bilirubin levels were decreased among normal (26%; $P=0.007$) and intermediate metabolizers (41%; $P<0.0001$) (Fig 4C). The slow metabolizers showed a decreasing trend (52%) with unconjugated bilirubin levels, but did not meet statistical significance probably due to small sample size.

***SLCO1B1* polymorphisms not associated with inter-individual variability in bilirubin levels**

There were no changes observed between total, conjugated, and unconjugated bilirubin levels across genotype-predicted phenotypes at screening or after completion of study (exit) (Supplemental Figure 1). Total bilirubin levels significantly decreased after the completion of efavirenz treatment in genotyping normal ($P<0.0001$) and intermediate ($P<0.0001$) metabolizers (Supplemental Figure 2A). Conjugated bilirubin levels were significantly reduced only among normal metabolizers ($P<0.01$) and unconjugated bilirubin levels were decreased in normal ($P<0.0001$) and intermediate ($P<0.05$) metabolizers (Supplemental Figure 2B and 2C). No changes were observed when stratifying UGT1A1 normalizers only (Supplemental Figure 3).

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Discussion

We found that variants in the *UGT1A1* gene, but not *SLCO1B1* genotypes, are associated with baseline bilirubin plasma concentrations, i.e., total bilirubin was significantly higher in slow metabolizer compared to normal and intermediate metabolizers. Slow metabolizers had slightly higher total bilirubin after efavirenz treatment, but the associations with genotype-predicted phenotypes did not reach a statistically significant level. While efavirenz significantly reduced total bilirubin concentrations in all genotypes, this effect was more pronounced in *UGT1A1* genotypic slow metabolizers, as there was a 41% decrease in total bilirubin levels versus a 29% and 35% decrease among normal and intermediate metabolizers, respectively. Certain drugs (e.g., atazanavir and indinavir) have been shown to increase bilirubin levels through inhibition of *UGT1A1* and this interaction has been associated with hyperbilirubinemia, jaundice, and premature discontinuation of treatment particularly in those with *UGT1A1**28 alleles (Rotger et al., 2005; Gammal et al., 2016). Our data suggests that *UGT1A1* genotype-dependent differential induction of *UGT1A1* activity by efavirenz may occur, although the sample size in the slow metabolizers is relatively small. If this finding is confirmed in a larger sample size, efavirenz interaction with *UGT1A1* substrates may be expected to depend on *UGT1A1* genotypes such that the magnitude of efavirenz interaction with substrates of *UGT1A1* would be greater in slow metabolizer, potentially necessitating dose adjustment when coadministered with efavirenz (e.g., in patients with Gilbert syndrome).

We found that chronic administration of efavirenz significantly reduced total, unconjugated and conjugated (glucuronides) bilirubin by 33%, 36% and 31%, respectively. These findings concur

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with our previous study showing a 30% decrease in total bilirubin levels by efavirenz (Metzger et al., 2014) and with those from other investigators where efavirenz was shown to decrease unconjugated and conjugated bilirubin by 42% and 26%, respectively, in a small number of healthy volunteers (Lee et al., 2012). Although the exact mechanisms whereby efavirenz reduces bilirubin levels remains unknown, a useful plausible insight can be obtained from a close look at the processes responsible for the hepatic disposition of unconjugated and conjugated bilirubin (Crawford et al., 1992; Bosma et al., 1995; Jedlitschky et al., 1997; Kadakol et al., 2000; van de Steeg et al., 2012; Sticova and Jirsa, 2013; Keppler, 2014) (see also Figure 1). Multiple transporters have been implicated in bilirubin hepatic disposition, but UGT1A1 and MRP2 appear to mediate the effect of efavirenz on unconjugated and conjugated bilirubin levels.

In vitro and/or *in vivo* evidence has shown efavirenz-mediated pregnane X receptor activation (Sharma et al., 2013) to induce *UGT1A1* and *MRP2* expression (Gwag et al., 2019) and efavirenz-mediated constitutively androgen receptor activation to induce *UGT1A1* expression (Meyer zu Schwabedissen et al., 2012). Considering that unconjugated bilirubin is a substrate of UGT1A1 (Crawford et al., 1992) and efavirenz substantially enhances the clearance of UGT1A1 substrates such as dolutegravir (Song et al., 2014), we believe that the observed decrease in unconjugated bilirubin by chronic administration of efavirenz is primarily due to induction of UGT1A1. Of note, the most potent inhibitor of UGT1A1 atazanavir results in unconjugated hyperbilirubinemia in HIV patients (Gammal et al., 2016), indicating that UGT1A1 is a rate limiting step in its hepatic clearance. Hepatic uptake of unconjugated bilirubin from blood into hepatocytes has been shown to be mediated in part via OATP1B1/3 transporters (Keppler et al., 2014) (Figure 1) and this was confirmed in humans where increased unconjugated bilirubin was

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noted in complete absence of OATP1B1/3 as is the case with Rotor syndrome (van de Steeg et al., 2012). However, associations of reduced function variants in the *SLCO1B1* gene with unconjugated bilirubin level were inconsistent, which included no significant associations (our present data; Hu and Tomlinson, 2012; Bae et al., 2019) or significant associations (Zhang et al., 2007). Although chronic administration of efavirenz significantly reduces plasma exposure the OATP1B1/3 and MRP2 substrate pravastatin (Gerber et al., 2005), efavirenz did not affect the exposure of a more selective OATP1B1/3 substrate pitavastatin (Malvestutto et al., 2014), further supporting that efavirenz interaction with OATP1B1/3, if any, is likely marginal. Together, these data suggest that the role of OATP1B1/3 in hepatic uptake of unconjugated bilirubin is apparent only under extreme transporter functional deficiencies (van de Steeg et al., 2012) and that efavirenz and variants in the *SLCO1B1* gene may not be an effective modulators of unconjugated bilirubin.

Efavirenz induction of UGT1A1-mediated metabolism of unconjugated bilirubin would have been expected to increase plasma bilirubin glucuronides, but we found the opposite, i.e., efavirenz treatment decreased bilirubin conjugates. Bilirubin glucuronides are good substrates of MRP2, MRP3 and OATP1B1/3 (Figure 1). Indeed, inherited OATP1B1/3 are associated with conjugated (and to some extent unconjugated) hyperbilirubinemia, as seen with Rotor-syndrome, whereas inherited deficiency in MRP2 results in conjugated hyperbilirubinemia as seen in human Dubin-Johnson syndrome (van de Steeg et al., 2012; Keppler 2014). As described above, the effect of efavirenz on OATP1B1/3 is likely marginal and, although efavirenz may show induction of mRNA of MRP3 in vitro (Weiss et al., 2009), the reuptake mechanism for bilirubin glucuronides by OATP1B1 is so efficient and rate limiting to have an impact on plasma

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concentrations of these metabolites. In fact, there appears no evidence that efavirenz may affect MRP3 clinically (Kharasch et al., 2012). While a small contribution of MRP3 or OATP1B1/3 cannot be fully excluded, we believe that a concomitant efficient induction of MRP2-mediated efflux transport to the bile by efavirenz leads to a net reduction in conjugated bilirubin. Of note, efavirenz significantly reduce plasma exposure of pravastatin (OATP1B1/3 and MRP2 substrate) (Gerber et al., 2005), while no such effect is observed on the exposure of pitavastatin (a selective OATP1B1/3 substrate) (Malvestutto et al., 2014), providing further evidence that the efavirenz-pravastatin is likely due to induction of MRP2.

Reduction of bilirubin levels by efavirenz may have clinical relevance. Mildly elevated serum bilirubin levels are associated with reduced risk for oxidative stress and cardiovascular disease for individuals with HIV and non-HIV infected patients. Elevated levels of unconjugated bilirubin observed due to variants in the *UGT1A1* gene (e.g., *UGT1A1* *28 in Gilbert syndrome) (Burchell and Hume, 1999) or concomitant use of drugs that inhibit UGT1A1 activity (Gammal et al., 2016) have been shown to reduce cardiovascular risks or markers of cardiovascular risks (Vitek et al., 2002; Lin et al., 2006; Estrada et al., 2016). There is clinical evidence that atazanavir (a clinical inhibitor of UGT1A1) containing HIV therapy reduces cardiovascular risk when compared to efavirenz-based regimen (Estrada et al., 2016; LaFleur et al., 2017). The possibility that the substantial reduction in serum bilirubin levels during chronic treatment with efavirenz or other inducers would diminish the favorable effect of bilirubin on oxidative stress and cardiovascular outcomes cannot be ruled out and needs further study.

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In summary, our data suggest that efavirenz is an effective inducer of *UGT1A1* (indicated by reduction in unconjugated bilirubin) and *MRP2* (indicated by significant changes in conjugated bilirubin). Efavirenz likely alters the disposition of UGT1A1 and MRP2 substrate drugs, potentially causing lack of efficacy or increased adverse effects, and endogenous compounds relevant in homeostasis. It follows that measurement of unconjugated and conjugated bilirubin during new drug development may provide mechanistic understanding regarding enzyme and transporters modulated. Our data indicate that *UGT1A1* genotype, but not *SLCO1B1* genotypes, predict serum bilirubin levels at baseline. Also, that efavirenz treatment somewhat abrogated *UGT1A1* genetic associations with bilirubin disposition. Although UGT1A1 or *SLCO1B1* genotype-dependent induction of bilirubin disposition was suggested, further studies are needed to confirm this trend in an adequate sample size. Lastly, even though *UGT1A1**80 and *28 are in strong LD ($r^2=0.96$), few subjects would be misclassified as *28 (2%) when *80 is used as a tag-SNP. Although genotyping for *28 is more accurate when feasible, genotyping for *80 can be used if assays for *28 are not available.

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Authorship Contributions

Participated in research design: Collins, Gufford, Pratt, Skaar, Desta

Conducted experiments: Collins, Metzger, Lu, Pratt, Medeiros

Contributed new reagents or analytic tools: Desta

Performed data analysis: Collins, Metzger, Gufford, Medeiros, Pratt, Desta

Wrote or contributed to the writing of the manuscript: Collins, Gufford, Pratt, Skaar, Desta

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Footnotes

This study was supported by funding from NIH/NIGMS F31-GM119401 (Collins), Clinical pharmacology Training Grant T32-GM008425 (Gufford), R01-GM078501 (Desta), R01-GM088076 (Skaar) and Indiana University School of Medicine Strategic Research Initiative (Pratt/Medeiros) and the IGNITE project grant (Pratt).

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Table 1: Demographics of Study Population

	N= 136
Age (range)	27 (18-50)
Weight (range)	73.3 (52.3-109)
BMI (range)	24.4 (17.8-32.2)
Male, N (%)	80 (59)
Ethnicity	
White, N (%)	89 (65.4)
Black, N (%)	23 (17)
Asian, N (%)	12 (8.8)
Other, N (%)	12 (8.8)

Continuous variables reported as average (range). Categorical variables reported as the count (percentage).

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Table 2: *UGT1A1* and *SLCO1B1* genotype counts and frequencies in population.

		Genotype	Count	Frequency
UGT1A1	rs887829	*1/*1	61	0.449
		*1/*80	62	0.456
		*80/*80	13	0.096
	rs8175347	*1/*1	60	0.441
		*1/*28	54	0.397
		*1/*36	1	0.007
		*1/*37	5	0.037
		*28/*28	12	0.088
		*28/*36	4	0.029
	rs4148323	*1/*1	135	0.993
		*1/*6	1	0.007
SLCO1B1	rs4149056	*1/*1	111	0.810
		*1/*5	17	0.124
		*5/*5	8	0.058
	rs2306283	*1/*1	38	0.279
		*1/*1b	54	0.397
		*1b/*1b	44	0.324

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Table 3: CPIC classification of *UGT1A1* and *SLCO1B1* phenotypes based on genotypes

		Genotypes	Count	Frequency
UGT1A1	Normal	*1/*1, *1/*36, *36/*36	60	0.441
	Intermediate	*1/*28, *1/*37, *36/*28, *1/*6	64	0.471
	Slow	*28/*28, *37/*37	12	0.088
SLCO1B1	Normal	*1/*1, *1/*1b, *1b/*1b	111	0.816
	Intermediate	*1/*5, *1/*15	17	0.125
	Slow	*5/*5, *15/*15	8	0.059

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Figure Legends

Figure 1: Bilirubin conjugation and transport in the liver. Unconjugated bilirubin (UB), a product of heme catabolism, is uptake transported into hepatocytes in part by OATP1B1/3 and unknown transported as well as by diffusion. In hepatocytes, UB undergo UGT1A1-mediated glucuronidation to mono- and bi-glucuronides. Conjugated bilirubin (CB) is then efflux-transported either to the bile by MRP2 and a substantial fraction efflux transported to the blood by MRP3 from where they are then subsequently reuptaken effectively back to the hepatocytes via OATP1B1/3. Efavirenz-mediated upregulation of UGT1A1 metabolism and efflux transport by MRP2 are shown by plus sign.

Figure 2: Multiple dose efavirenz effect on bilirubin levels irrespective of *UGT1A1* genotype-predicted phenotype. *Y-axis:* Plasma levels of total, conjugated and unconjugated bilirubin (mg/dL) measured before (Screening) and after (Exit) efavirenz treatment (*X-axis*). Total (n=133), conjugated (n=65) and unconjugated bilirubin levels (n=67). *** $P<0.001$, **** $P<0.0001$

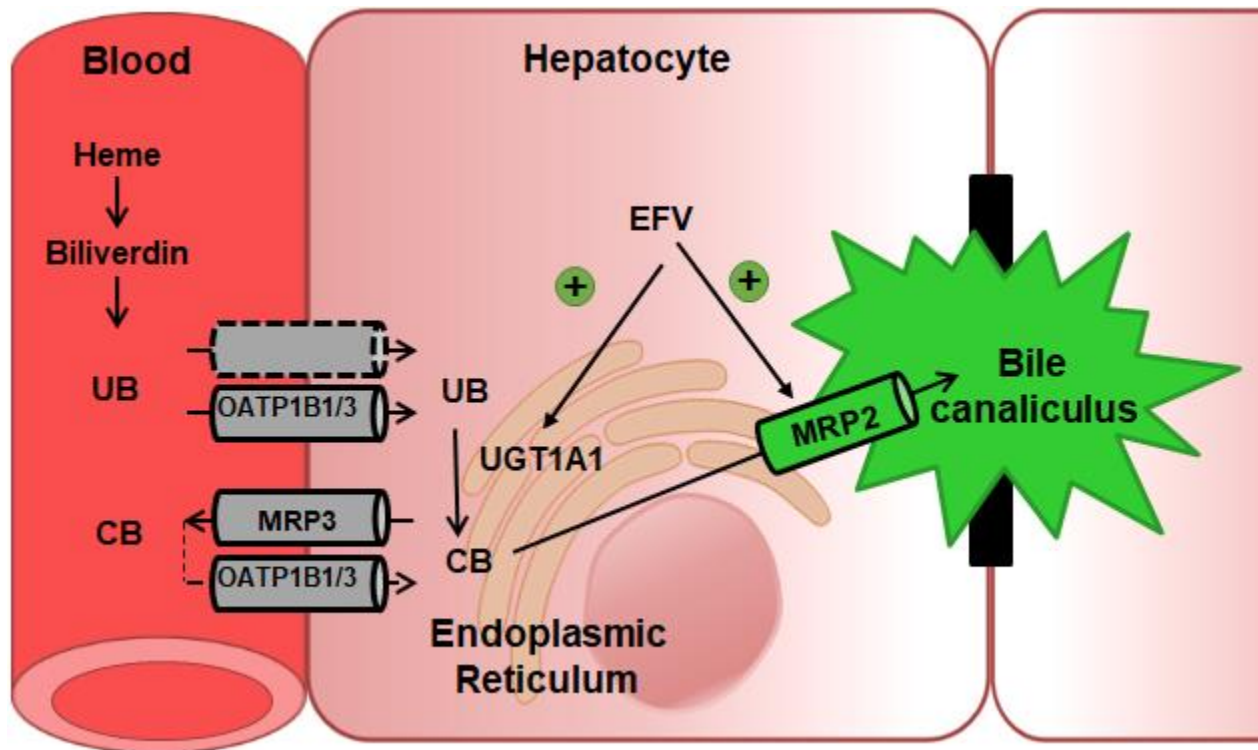
Figure 3: Association of *UGT1A1* genotyped-predicted phenotype with bilirubin levels before and after efavirenz treatment. Total bilirubin levels screening (**A**) and exit (**B**); conjugated bilirubin levels screening (**C**) and exit (**D**); and unconjugated bilirubin levels screening (**E**) and exit (**F**). *Y-axis:* Bilirubin levels (mg/dL). Categorization of *UGT1A1* genotypes predicted phenotypes (*X-axis*) was performed according to Table 3. Number of individuals by panel: **A.** 61, 62, 12; **B.** 59, 62, 12; **C.** 31, 33, 4; **D.** 30, 32, 4; **E.** 32, 33, 4; **F.** 30, 33, 4. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

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Figure 4: Multiple dosing efavirenz effect on bilirubin levels by *UGT1A1* genotype-predicted phenotype. Plasma levels of total, conjugated, and unconjugated bilirubin (mg/dL) measured before (Screen) and after (Exit) efavirenz treatment. Subjects were stratified by *UGT1A1* metabolizer phenotype according to Table 3. Total (n=59, 62, 12), conjugated (n=29, 32, 4) and unconjugated bilirubin levels (n=30, 33, 4), normal, intermediate, slow metabolizers, respectively. ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$

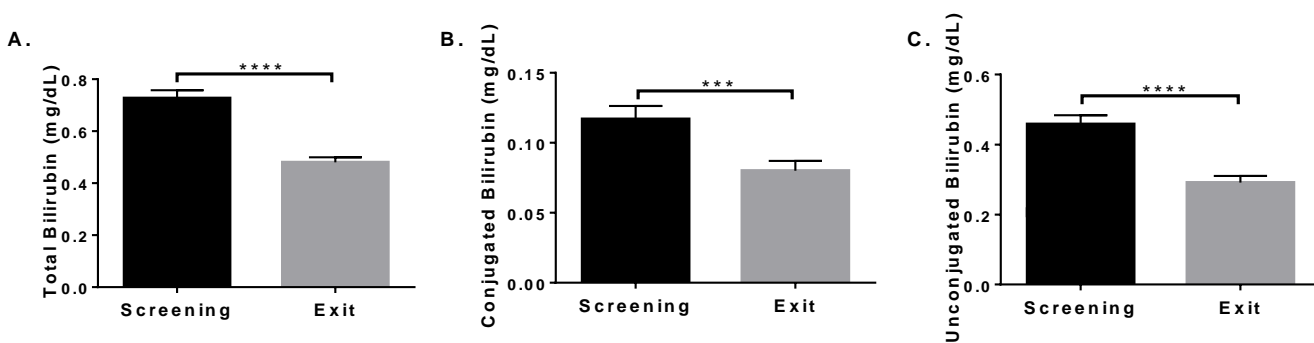
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Figure 1



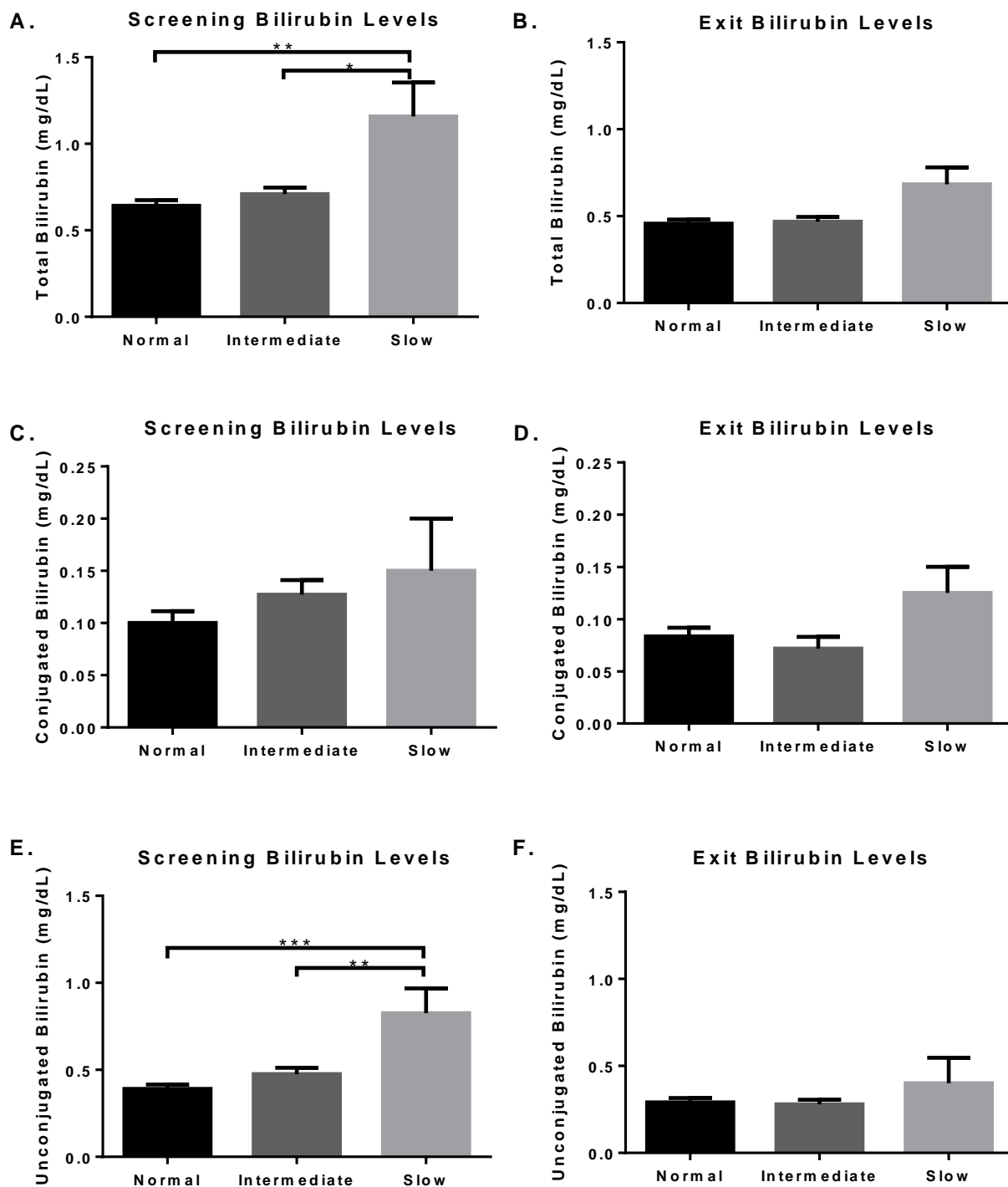
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Figure 2



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Figure 3



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Figure 4

