Effects of CYP2B6 G516T polymorphisms on plasma efavirenz and nevirapine levels when co-administered with rifampicin in HIV/TB co-infected Thai adults

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Abstract

Background: Cytochrome P450 2B6 (CYP2B6) metabolizes efavirenz and nevirapine, the major core antiretroviral drugs for HIV in Thailand. Rifampicin, a critical component of tuberculosis (TB) therapy is a potent inducer of CYP enzyme activity. Polymorphisms of CYP2B6 and CYP3A4 are associated with altered activity of hepatic enzyme in the liver and pharmacokinetics resulting in treatment efficacy. This study aimed to investigate whether CYP2B6 or CYP3A4 polymorphisms had effects on plasma efavirenz and nevirapine concentrations when co-administered with rifampicin in HIV/TB co-infected Thai adults.

Results: We studied 124 rifampicin recipients with concurrent HIV-1/TB coinfection, receiving efavirenz (600 mg/day) (n = 65) or nevirapine (400 mg/day) (n = 59) based antiretroviral therapy (ART). The frequencies of GG, GT and TT genotypes of CYP2B6-G516T were 38.46%, 47.69% and 13.85% in efavirenz group and 44.07%, 52.54% and 3.39% in nevirapine group, respectively. The mean 12-hour post-dose plasma efavirenz concentration in patients with TT genotype at weeks 6 and 12 of ART and 1 month after rifampicin discontinuation (10.97 ± 2.32, 13.62 ± 4.21 and 8.48 ± 1.30 mg/L, respectively) were significantly higher than those with GT (3.43 ± 0.29, 3.35 ± 0.27 and 3.21 ± 0.22 mg/L, respectively) (p < 0.0001) or GG genotypes (2.88 ± 0.33, 2.45 ± 0.26 and 2.08 ± 0.16 mg/L, respectively) (p < 0.0001). Likewise, the mean 12-hour post-dose plasma nevirapine concentration in patients carrying TT genotype at weeks 6 and 12 of ART and 1 month after rifampicin discontinuation (14.09 ± 9.49, 7.94 ± 2.76 and 9.44 ± 0.17 mg/L, respectively) tended to be higher than those carrying GT (5.65 ± 0.54, 5.58 ± 0.48 and 7.03 ± 0.64 mg/L, respectively) or GG genotypes (5.42 ± 0.48, 5.34 ± 0.50 and 6.43 ± 0.64 mg/L, respectively) (p = 0.003, p = 0.409 and p = 0.448, respectively). Compared with the effects of CYP2B6-G516T genotype, we could observe only small effects of rifampicin on plasma efavirenz and nevirapine levels. After 12 weeks of both drug regimens, there was a trend towards higher percentage of patients with CYP2B6-TT genotype who achieved HIV-1 RNA levels <50 copies/mL compared to those with GT or GG genotypes. This is the first report to demonstrate the effects of CYP2B6 G516T polymorphisms on plasma efavirenz and nevirapine concentrations when co-administered with rifampicin in HIV/TB co-infected Thai adults.

Conclusions: CYP2B6-TT genotype had impact on plasma efavirenz and nevirapine concentrations, while rifampicin co-administration had only small effects.

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Background

Tuberculosis (TB) is the most common opportunistic infections in human immunodeficiency virus (HIV) infected individuals, accounting for more than 30% in Thailand, and up to 50% of them die during treatment [1]. The mortality is reduced in HIV-TB co-infected patients who have started the combination antiretroviral therapy after diagnosis of TB [2]. Concomitant administration of highly active antiretroviral therapy (HAART) and anti-TB medications is often complicated due to the drug-drug interaction and the adverse effect profile. Efavirenz and nevirapine based HAART regimens have mostly recommended to use as components of first-line antiretroviral drug regimens worldwide [3]. As efavirenz and nevirapine are potent non-nucleoside reverse transcriptase inhibitors (NNRTIs), they are the preferable option for initial antiretroviral treatments (ART) in HIV/TB co-infection. Rifampicin is a critical component of TB therapy while it is a potent inducer of cytochrome P450 (CYP) enzyme activity [4]. The available pharmacokinetic data showed that rifampicin reduced the plasma concentration of efavirenz and nevirapine of 13-25% and 40%, respectively [5-7]. Recently, efavirenz was shown in vitro to be primarily metabolized by hepatic CYP2B6, with minor contributions from CYP3A4 and CYP2A6 [4,8]. While rifampicin is an inducer of CYP3A4, nevirapine induces more CYP2B6 than CYP3A4 [9]. Nevirapine was also shown to be principally metabolized by CYP3A4 and CYP2B6 [10]. CYP2B6 and CYP3A4 genotypes are evidenced to be associated with altered activity of hepatic enzyme in the liver and pharmacokinetics that may influence efficacy of treatment, since rifampicin causes decrease in efavirenz and nevirapine concentrations [11-13].

The CYP2B6 and CYP3A4 genes are highly polymorphic [14] and are subject to pronoun interindividual variability in expression and activity. A single nucleotide polymorphism (SNP) at position 516 on the CYP2B6 gene has been widely reported to play an important role in the metabolism of antiretroviral drugs [15-18]. This CYP2B6 genetic variant affects the efavirenz and nevirapine pharmacokinetics [16,19,20] and associated with clinical response to nevirapine-containing regimens in children [16]. Significant advances have led to a greater understanding of interactions between genetic and host factors that influence the efficacy and toxicity of efavirenz [19,21]. However, the findings from one population may not be generalised to other populations due to the ethnic differences in drug effect and body weight of the patients. In Thailand, it has been recently reported that CYP2B6-G516T polymorphism significantly affected the drug metabolism of efavirenz in HIV-infected Thai children [22], while its impact on nevirapine concentrations was less pronounced after intrapartum single-dose nevirapine in HIV-infected mothers [23]. As efavirenz or nevirapine-based HAART is being used as the main therapy in Thailand, however, limited information was obtained so far among various Thai population regarding the influence of host genetic polymorphism on these drug levels especially nevirapine when co-administered with rifampicin which is essential for optimization of ARV dosage or drug-drug interaction. Therefore, the main objective of the present study is to investigate whether CYP2B6 and CYP3A4 polymorphisms could influence the plasma efavirenz and nevirapine levels when co-administered with rifampicin in HIV/TB infected Thai adults. The evaluation of clinical and immunological outcomes was also aimed.

Methods

Patients

One hundred and twenty four rifampicin recipients with concurrent HIV-1/TB coinfection were studied. Sixty-five of them received efavirenz (600 mg/day) based ART while 59 received nevirapine (400 mg/day) based ART. Initially, 142 patients were recruited for the study on a randomized control trial to compare the efficacy of efavirenz and nevirapine among HIV-infected patients receiving rifampicin at Bamrasnaradura Infectious Diseases Institute (BIDI), Nonthaburi since December 2006 [24]. They are ARV naïve with active tuberculosis and received rifampicin containing anti-TB regimens for 4-6 weeks prior to enrolment. The patients received oral lamivudine (150 mg) and stavudine (30 mg for those who weighed ≤ 60 kg and 40 mg for those who weighed >60 kg) every 12 hours. They were randomized to receive either efavirenz 600 mg at bedtime while fasting or nevirapine 200 mg every 12 hours after 2 weeks at a starting dose of 200 mg every 24 hours. The dosage of rifampicin was 450 mg/day for patients who weighed ≤ 50 kg and 600 mg/day for those who weighed >50 kg. The anti-TB drug regimen was isoniazid, rifampicin, ethambutol and pyrazinamide for the first two months, followed by isoniazid and rifampicin for the subsequent 4-7 months. Among 142 patients recruited, 25 patients (9 in the efavirenz group and 16 in the nevirapine group) failed to continue the study because of hepatitis (2 cases in the nevirapine group), skin rash (3 in the efavirenz group and 2 in the nevirapine group), death (2 in the efavirenz group and 6 in the nevirapine group), transfer to the other hospital (1 in the nevirapine group), or lost to follow up (4 in the efavirenz group and 5 in the nevirapine group). In the present study, we analyzed 124 patients who have a complete data set of plasma drug levels at week 6 and 12 of ART and 1 month after rifampicin discontinuation. The study was approved by Institutional Ethics Committees of Bamrasnaradura Infectious Diseases Institute and the Ministry
of Public Health, Thailand and the written informed consents were obtained from all participants.

**Blood samples**
EDTA bloods were collected from patients for SNP genotyping, CD4 T cell counts and HIV-1 viral load. Lithium heparinized bloods were collected after 12 hours of drug administration (C12) at weeks 6 and 12 of ART and after rifampicin discontinuation for 1 month for analysis of plasma efavirenz and nevirapine concentrations. The plasma were separated by centrifugation at 1800 g for 20 minutes and stored at -20°C.

**SNP genotyping of CYP2B6 and CYP3A4**
The genomic DNA was extracted by using QIAamp DNA blood Mini kit (QIAGEN, Hilden, Germany) and stored at -20°C for SNP genotyping. Genotyping of allelic variants in CYP2B6 and CYP3A4 were carried out by real-time PCR using the allelic-specific fluorogenic 5’ nuclease chain reaction assay by ABI PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA) as described previously [15]. Seven SNPs were genotyped: 4 SNPs of CYP2B6-516T, -C777A, -A415G and -C1459T and 3 SNPs of CYP3A4-T566C, -T878C and C1088T. Each 25 µl PCR mixture contained 20 ng of genomic DNA, 900 nM primers, 200 nM TaqMan minor groove binder (MGB) probes and 12.5 µl TaqMan universal PCR master mix. The thermal cycler program was set up at 95°C for 10 minutes, and then repeated 40 cycles with 95°C for 15 seconds and 60°C for 1 minute. The plate was read by the allelic discrimination settings. The SNP assay was set up using SDS, version 1.3.0 as an absolute quantification assay. Post-assay analysis was done by using SDS software.

**Determination of plasma efavirenz and nevirapine concentration**
Plasma efavirenz and nevirapine concentrations were measured by reverse phase high performance liquid chromatography (HPLC) method at the HIV-Netherlands-Australia-Thailand (HIV-NAT) Research Pharmacokinetic Laboratory, Chulalongkorn Medical Research Center (Bangkok, Thailand). HPLC was performed in accordance with the protocol developed by Department of Clinical Pharmacology, University Medical Center Nijmegen (Nijmegen, the Netherlands) [25].

**CD4 T lymphocyte counts and plasma HIV-1 RNA quantitation**
The CD4 T lymphocyte counts were done at baseline and every 12 weeks after initiation of antiretroviral treatment by flow cytometry using monoclonal antibodies with three colors reagent (TriTEST, Becton Dickinson BioSciences, USA) and analyzed by FACScan flow cytometer (Becton Dickinson BioSciences, USA.). Plasma HIV-1 RNA was determined by RT-PCR at baseline and every 12 weeks after initiation of ART and quantified using the COBAS Amplicor, version 1.5 (Roche Diagnostics, USA). The lower detection limit for HIV-1 RNA level is 50 copies/mL.

**Statistical analysis**
The different genotypes in relation to plasma drug levels were analysed by SPSS software version 14.0 (ID 5038562) (SPSS Inc., Chicago, IL, USA). If unpaired one-way analysis of variance (ANOVA) was significant (p < 0.05), then post hoc Scheffe’s F test was applied for multiple comparison. When plasma drug levels of different time points were compared, paired T test was used. The CD4 T cell counts and HIV-1 viral load in patients carrying different genotypes were compared by Kruskal-Wallis test. A difference in proportion of patients who achieved plasma HIV-1 RNA < 50 copies/ml at week 12 of ART was evaluated by Chi square or Fisher’s exact test. A p value of < 0.05 was considered statistically significant.

**Results**
**Patient characteristics**
The baseline characteristics of patients are shown in Table 1. All 124 patients were ethnically Thai and among these, 64.6% and 67.8% were male in efavirenz and nevirapine groups, respectively. The patients had the mean ages of 35.89 ± 8.17 and 38.03 ± 8.60 years and the mean body weights of 53.30 ± 9.79 and 54.39 ± 9.39 kg in efavirenz and nevirapine groups, respectively. Similar levels of laboratory parameters including alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin and direct bilirubin were seen in both patient groups. However, the levels of alkaline phosphatase among patients carrying TT genotype in efavirenz group were higher than those carrying GG or GT genotypes, but this difference was not statistically significant (p = 0.085). The median (interquartile range, IQR) CD4 T lymphocyte counts were similar in both groups. In nevirapine treatment group, the log number of plasma HIV-1 viral load among patients carrying GG, GT and TT genotypes seem to be significantly different (p = 0.041).

**Frequencies of CYP2B6 and CYP3A4 genetic polymorphisms**
Seven SNPs: 4 SNPs of CYP2B6- G516T, -C777A, -A415G and -C1459T and 3 SNPs of CYP3A4-T566C, -T878C and -C1088T were genotyped. For CYP2B6-G516T, 38.46% (25/65) of GG genotype (wild-type), 47.69% (31/65) of GT genotype (heterozygous mutant) and 13.85% (9/65) of TT genotype (homozygous
Table 1 Baseline characteristics of 124 HIV/TB co-infected patients with CYP2B6-G516T genotypes in efavirenz and nevirapine groups.

| Baseline characteristics       | Efavirenz group (n = 65) | Nevirapine group (n = 59) |
|-------------------------------|--------------------------|---------------------------|
|                               | GG GT TT p-value         | GG GT TT p-value          |
| Sex Male: Female              | n = 25 n = 31 n = 9     | n = 26 n = 31 n = 2       |
| Age years, mean (SD)          |                          |                          |
|                               | 16.9 (8.08)              | 17.9 (8.08)               |
|                               | 21.10 (8.82)             | 22.9 (8.82)               |
|                               | 5.4 (6.63)               | 3.5 (6.63)                |
|                               | 0.795 (6.63)             | 0.729 (6.63)              |
| Body weight kg, mean (SD)     |                          |                          |
|                               | 52.9 (1.89)              | 54.6 (2.06)               |
|                               | 53.94 (3.04)             | 54.7 (1.52)               |
|                               | 52.22 (6.06)             | 46.5 (6.5)                |
|                               | 0.872 (6.5)              | 0.872 (6.5)               |
| Alkaline phosphatase, U/L, mean (SD) | 149.2 (169.1) | 150.25 (28.9) |
|                               | 137.1 (68.45)            | 113.97 (11.1)             |
|                               | 233.9 (10.21)            | 125 (4)                   |
|                               | 0.085 (4)                | 0.085 (4)                 |
| Aspartate aminotransferase U/L, mean (SD) | 32.8 (3.32) | 48.54 (7.31) |
|                               | 40.48 (10.21)            | 35.58 (2.99)              |
|                               | 43.22 (10.21)            | 26 (1)                    |
|                               | 0.202 (1)                | 0.202 (1)                 |
| Alanine aminotransferase, U/L, mean (SD) | 27.0 (3.05) | 29.81 (3.95) |
|                               | 28.55 (8.89)             | 27.94 (3.57)              |
|                               | 31.22 (8.89)             | 23.5 (5.5)                |
|                               | 0.821 (8.89)             | 0.821 (8.89)              |
| Total bilirubin, mg/dL, mean (SD) | 4.9 (4.34) | 2.97 (2.4)  |
|                               | 0.56 (0.05)              | 1.13 (0.57)               |
|                               | 0.43 (0.07)              | 0.6 (0.1)                 |
| Direct bilirubin, mg/dL, mean (SD) | 0.45 (0.14) | 0.28 (0.047) |
|                               | 0.37 (0.01)              | 0.52 (0.199)              |
|                               | 0.21 (0.05)              | 0.30 (0.1)                |
| CD4 count, cells/μL, median (IQR) | 41 (18-102) | 35.5 (23.5-97) |
|                               | 54 (24-120)              | 45 (25-113)               |
|                               | 67 (12.5-168)            | 30.5 (23)                 |
|                               | 0.818 (23)               | 0.595 (23)                |
| Log Plasma HIV-1 viral load, median (IQR) | 5.90 (3.57-6.0) | 5.86 (5.46-6.0) |
|                               | 5.93 (5.39-6.0)          | 5.60 (5.41-5.81)          |
|                               | 5.64 (5.30-6.0)          | 5.80 (Q1 = 5.59)          |

* Statistically significant by Kruskal-Wallis test. SD: standard deviation. IQR: interquartile range.

Table 1: Baseline characteristics of 124 HIV/TB co-infected patients with CYP2B6-G516T genotypes in efavirenz and nevirapine groups. The table presents the baseline characteristics for both the efavirenz and nevirapine groups, including sex, age, body weight, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total bilirubin, direct bilirubin, CD4 count, and log plasma HIV-1 viral load. The data are presented as mean (standard deviation) for continuous variables and median (interquartile range) for log plasma HIV-1 viral load. The p-values are provided for statistical significance.

CYP2B6-G516T and CYP3A4-T878C genetic polymorphisms and plasma efavirenz and nevirapine concentrations

Among 4 SNPs of CYP2B6-G516T, -C777A, -A415G and -C1459T being evaluated, the frequencies of wild-type, heterozygous mutant and homozygous mutant were well distributed only in CYP2B6-G516T polymorphism, therefore, the analysis of this gene polymorphism was further done in relation to plasma efavirenz and nevirapine levels. The mean plasma efavirenz concentration in patients with homozygous TT genotype at weeks 6 and 12 of ART and 1 month after rifampicin discontinuation (10.97 ± 2.32, 13.62 ± 4.21 mg/L and 8.48 ± 1.30 mg/L, respectively) were significantly higher than those with GT genotype (5.42 ± 0.48, 5.34 ± 0.50 and 6.43 ± 0.64 mg/L, respectively) or GG genotype (7.94 ± 2.76 and 9.44 ± 0.17 mg/L, respectively) tended to be higher than those with GT genotype (5.42 ± 0.48, 5.34 ± 0.50 and 6.43 ± 0.64 mg/L, respectively) or GG genotype (2.88 ± 0.33, 2.45 ± 0.26 and 2.08 ± 0.16 mg/L, respectively) (p < 0.0001) (Figure 1a, b, c). Similar results were found in nevirapine group (Figure 1d, e, f) in that the mean plasma drug concentration of patients with TT genotype at weeks 6 and 12 of ART and 1 month after rifampicin discontinuation (14.09 ± 9.49, 7.94 ± 2.76 and 9.44 ± 0.17 mg/L, respectively) tended to be higher than those with GT genotype (3.43 ± 0.29, 3.35 ± 0.27 mg/L and 3.21 ± 0.22 mg/L, respectively) or GG genotype (2.88 ± 0.33, 2.45 ± 0.26 and 2.08 ± 0.16 mg/L, respectively) (p < 0.0001) (Figure 1a, b, c).
discontinuation (3.5 ± 2.67 mg/L) were significantly lower than those at week 6 (4.26 ± 3.96 mg/L) (p = 0.043) and tended to be lower than those at week 12 (4.42 ± 5.97 mg/L) (p = 0.133). In contrast, plasma nevirapine levels at 1 month after rifampicin discontinuation (6.84 ± 3.4 mg/L) were significantly higher than those at week 6 (5.83 ± 3.6 mg/L, p = 0.034) and those at week 12 (5.56 ± 2.63 mg/L, p < 0.001). The reason for these discrepant results on effects of rifampicin on plasma efavirenz and nevirapine levels is not clear at present. Further studies including evaluation of plasma drug levels at time points other than 12-hour post-dose would be thus necessary. Nevertheless, we at least can conclude that the magnitude of effects on plasma...
efavirenz and nevirapine levels by rifampicin was much smaller than that by CYP2B6 516 TT genotype.

With respect to CYP3A4, the analysis was done in only CYP3A4-T878C, since there was no variation at the CYP3A4-T878C and -C1088T in our study subjects. The results showed that the mean plasma efavirenz concentration at weeks 6 and 12 of ART and 1 month after rifampicin discontinuation were 4.00 ± 0.42, 4.20 ± 0.72 and 3.48 ± 0.34 mg/L, respectively, in patients with homozygous TT genotype and 9.62 ± 6.33, 8.97 ± 6.33 and 3.87 ± 1.69 mg/L, respectively, in those with heterozygous TC genotype. Similarly, the mean plasma nevirapine concentration at weeks 6 and 12 of ART and 1 month after rifampicin discontinuation were 5.50 ± 0.34 and 6.80 ± 0.45 mg/L, respectively, in patients with homozygous TT genotype, and 4.8, 8.69 and 9.12 mg/L, respectively, in one patient with heterozygous mutant TC genotype. Although there was a trend towards higher plasma drug levels in patients with heterozygous mutant TC genotype, appropriate statistical evaluation of this difference was difficult due to small numbers of heterozygous mutant TC.

Discussion

This is the first report to demonstrate the effects of CYP2B6-G516T and CYP3A4-T878C polymorphisms on plasma efavirenz and nevirapine concentrations in rifampicin-treated HIV/TB co-infected Thai adults. The results indicated that the wide interindividual variability of efavirenz concentrations is strongly influenced by CYP2B6-516TT genotype by the finding of significantly higher plasma efavirenz concentration at weeks 6 and 12 of ART and 1 month after rifampicin discontinuation compared to those with GT or GG genotype. Likewise, it seems to be that this CYP2B6-516TT would also influence nevirapine concentrations, although it was less pronounced probably due to the small samples size of homozygous mutant TT in our sample set. The present results were in line with the previous report on efavirenz pharmacokinetics when co-administration with rifampicin in HIV/TB co-infected Indian [26,27] and Ghana patients [28] in that plasma efavirenz was highest in patients with CYP2B6-516TT genotype when compared to those with GT or GG genotypes. While the heterozygous TC mutant in CYP3A4-T878C in this study seems to have some effects on plasma drug concentrations in patients at weeks 6 and 12 of ART and 1 month after rifampicin discontinuation in both efavirenz and nevirapine groups, further statistical analysis was not done due to the relatively less variation of CYP3A4 among Thai adults in this study. Further investigation should include a larger sample size with varying genotypes in order to draw a definite conclusion on the effect of CYP3A4 variations.

In this study, the frequency of CYP2B6-G516T among 124 Thai adults was 8.9%, which was close to that of our recent study on 237 HIV-infected Thai adults with different rate of CD4 T cell recovery after ARV treatment (9.7%) (submitted for publication) and slightly lower than what has been reported in Thai children (11%) [22]. Comparing to the other ethnic groups, it was higher than those of Japanese (3.3%) [15] and Caucasian (6%) [14], but lower than that of African-American (20%) [21] or African population (23%) [28,29]. Although the frequencies of CYP2B6-G516T were different among populations or ethnicity, the pharmacogenetic studies reported so far in HIV patients
demonstrated that CYP2B6 516TT was definitely associated with plasma efavirenz concentration [15,19,21,29,30]. The findings of CYP2B6 516TT genotype in the present study support its effect on plasma efavirenz concentration in different ethnic group and gave additional information of this SNP on nevirapine based-ART when co-administered with rifampicin. The recent pharmacogenetic study in HIV patients co-administered with efavirenz and rifampicin demonstrated that patients carrying TT genotype had significantly higher mean plasma efavirenz but lower oral clearance [28], indicating that rifampicin does not fully reverse the poor metabolizer phenotype and that TT genotype can be used to identify poor metabolizers of efavirenz even

![Figure 2](Figure.png)

**Figure 2** Median CD4 T cell counts among HIV/TB adults with CYP2B6-G516T polymorphism at different time points. (Black diamond) GG genotype, (Black square), GT genotype, (Black triangle) TT genotype in efavirenz (a) and nevirapine groups (b) at baseline, 12, 24, 36 and 48 weeks of ART.

**Table 2** Number of patients with plasma HIV-1 RNA < 50 copies/ml at week 12 of ART.

|          | Efavirenz group (N = 65) | Nevirapine group (N = 59) |
|----------|--------------------------|---------------------------|
|          | CYP2B6-G516T              |                           |
|          | GG                       | GT                        | TT                        | p-value* | GG                       | GT                        | TT                        | p-value** |
| No. of patients (%) | 17 (68) | 24 (77.42) | 8 (88.89) | 0.430 | 15 (70.97) | 23 (70.97) | 2 (100) | 0.288 |

* Chi-square test  
** Fisher's exact test
in patients co-administrated with rifampicin. Consis-
tently, the present results also indicated that rifampicin
coadministration in HIV/TB infected patients did not
significantly alter plasma efavirenz and nevirapine levels
in patients with TT genotype (p > 0.05). Other possible
factors that might affect the plasma drug levels could be
excluded since they were carefully controlled.

Although rifampicin can cause the decrease in NNRTI
concentrations, the mean plasma efavirenz and nevira-
pine concentrations in all studied patients with TT, GT
and GG genotypes had plasma drug levels above the
minimum recommendation (1 mg/L for efavirenz and
3.4 mg/L for nevirapine). One important conclusion
from our recent prospective and randomized clinical
trial in patients with concurrent HIV/TB receiving
rifampicin [24] is that the standard dosage of efavirenz
600 mg or nevirapine 400 mg per day and co-adminis-
tration with rifampicin was adequate for HIV-1 suppres-
sion, however, variation in the plasma drug levels in
some patients were found, which might be due to the
genetic variations among individuals. Although we
reported recently that high body weights of the patients
were associated with a low efavirenz C12 at weeks 6 and
12 of ART [31], the present results demonstrated that
the body weights did not differ among patients with dif-
ferent genotypes of CYP2B6 516TT polymorphism. The
present results thus demonstrated that rifampicin has
very small effects on efavirenz and nevirapine plasma
drug. The advantage of our present study over previous
studies is that plasma efavirenz and nevirapine concen-
trations during co-administration of rifampicin could be
compared with those without rifampicin after complet-
ing TB drug treatment.

In general, the high plasma efavirenz and nevirapine
levels could lead to the adverse effect such as rash,
hepatitis, and neuropsychological toxicity [32,33]. In
order to reduce such adverse effects, several studies
attempted to test the feasibility of genotype-based dose
reduction of efavirenz in African-American [34] and
Japanese HIV infected patients [35] and showed that
efavirenz dose reduction is feasible and can reduce ef-
avirenz-associated central nervous system symptoms
in homozygotes of CYP2B6-G516T. Although patients with
CYP2B6-516TT in our cohort had obviously high
plasma efavirenz levels at all time points and certain
degree of central nervous system and psychiatric mani-
festations, they were all well tolerated with the adverse
effects. The adverse drug events have not recorded in
nevirapine based treatment probably due to the limited
number of patients with homozygous TT. Since there
were 7 cases who could not complete the study due to
side effects [24] it is necessary to determine CYP2B6
G516T genotypes of these individuals in order to know
whether CYP2B6-516TT homozygote in Thailand were
all well tolerated with the adverse effects of efavirenz
and nevirapine.

With respect to possible correlation of the variations
in plasma efavirenz and nevirapine levels with the treat-
ment outcome, our results indicated that the patients
with CYP2B6 516TT genotype had a higher frequency
of viral load suppression at week 12 of ART than those
with GT and GG genotype. The CD4 T cell counts
increased after treatment at all time points which were
 correlated with HIV-1 viral load reduction. When the
effect of different CYP2B6-G516T genotypes was ana-
ysed, no difference was observed among patients with
TT, GT and GG genotypes in both efavirenz and nevira-
pine groups. Collectively, it is indicated that the efavir-
enz and nevirapine-based ART co-administered with
rifampicin are well correlated with virological and
immunological outcomes in patients undergoing treat-
ment for HIV and TB.

In summary, the CYP2B6 and CYP3A4 polymorphisms
were analysed, for the first time, in HIV/TB co-infected
Thai adults receiving efavirenz and nevirapine based-
ART co-administered with rifampicin and the results
indicated that only 516G>T in CYP2B6 gene, but not
CYP3A4 gene polymorphism, gave the significant effects
on plasma drug levels. Only small effects of rifampicin
on efavirenz and nevirapine plasma concentration were
observed. However, for further investigation, other SNPs
such as CYP2B6 T983C or T346C-CYP2B6 haplotypes
which were shown to influence the NNRTI plasma drug
levels [23,36,37] should be taken into account and larger
sample size with varying genotypes should be included.

Conclusions
CYP2B6-516TT genotype had effects on both the plasma
efavirenz and nevirapine concentrations in HIV/TB
patients when co-administered with rifampicin. The
information might be useful for better treatment of
patients with HIV or HIV/TB.

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Authors’ contributions
SU, SL, WM, NW, TK, SK participated in the study design. SU performed genotyping. CD4 counts and HIV-1 viral load determination, analysed the data and drafted the manuscript. EEN and NW took part in genotyping. SL and WM coordinated the study. TS and SK revised and finalised the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare that they have no competing interests.

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References
1. Cain RP, Anekthananon T, Burapat C, Akksilp S, Mankhatitham W, Srinak C, Uttayamakul et al. Impact of CYP2B6 polymorphism on hepatic efavirenz metabolism in vitro. Pharmacogenomics 2007, 8:547-558.
2. Pinzani V, Faucherre V, Peyriere H, Blayac JP. Methadone withdrawal symptoms with nevirapine and efavirenz. Ann Pharmacother 2000, 34:405-407.
3. Erickson DA, Mather G, Trager WF, Levy RH, Keims JJ. Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. Drug Metab Dispos 1999, 27:1488-1495.
4. Cohen K, Grant A, Dandara C, McIlroy H, Goemaere E, Smith P, Maertens G. Effect of rifampin-based antitubercular therapy and the cytochrome P450 2B6 516G>T polymorphism on efavirenz concentrations in adults in South Africa. Antivir Ther 2009, 14:687-695.
5. Cabrera SE, Santos D, Valverde MP, Dominguez-Gil A, Gonzalez F, Luna G, Garcia MJ. Influence of the cytochrome P450 2B6 genotype on population pharmacokinetics of efavirenz in human immunodeficiency virus patients. Antimicrob Agents Chemother 2009, 53:2791-2798.
6. Cohen K, van Osten G, Bouille A, McIlroy H, Goemaere E, Smith P, Maertens G. Effect of rifampin-based antitubercular therapy on nevirapine plasma concentrations in South African adults with HIV-associated tuberculosis. J Antimicrob Chemother 2009, 61:389-393.
7. Lang T, Klein K, Fischer J, Nussler AK, Neuhapf U, Eichellbaum M, Schwab M, Zanger UM. The genetic polymorphism of the cytochrome P450 2B6 gene and its expression in human liver. Pharmacogenetic Pharmacogenomics 2001, 11:399-415.
8. Tsuchiya K, Gatanaga H, Tachikawa N, Teruya K, Kikuchi Y, Yoshino M, Kuhnawahanta T, Shiraoka T, Kimura S, Oka S. Homozygous CYP2B6 *6 (Q127H and K262R) correlates with high plasma efavirenz concentrations in HIV-1 patients treated with standard efavirenz-containing regimens. Biochem Biophys Res Commun 2004, 319:132-136.
9. Saitho A, Saies E, Capparelli E, Aweke F, Kovaci A, Burchett SK, Wania A, Nachman S, Fenton T, Specter SA. CYP2B6 genetic variants are associated with nevirapine pharmacokinetics and clinical response in HIV-1-infected children. AIDS 2007, 21:2191-2199.
10. Powers V, Ward J, Gompels M. CYP2B6*16T6 genotyping in a UK cohort of HIV-positive patients: polymorphism frequency and influence on efavirenz discontinuation. HIV Med 2009, 10:202-203.
11. Haas DW, Gebretsadik T, Mayo G, Menon UN, Acosta EP, Shintani A, Floyd M, Stein CM, Wilkinson GR. Associations between CYP2B6 polymorphisms and pharmacokinetics after a single dose of nevirapine or efavirenz in African americans. J Infect Dis 2009, 199:872-880.
12. King J, Abeg JA. Clinical impact of patient population differences and genomic variation in efavirenz therapy. AIDS 2008, 22:1909-1717.
13. Saitho A, Fletcher CV, Brundage R, Alvero C, Fenton T, Hsi a K, Specter SA. Efavirenz pharmacokinetics in HIV-1-infected children are associated with CYP2B6*1516T6 polymorphism. J Acquir Immune Defic Syndr 2007, 45:280-285.
14. Haas DW, Ribaudo HI, Kim RB, Tierney C, Wilkinson GR, Guilick RM, Clifford DB, Hulgan T, Marzolini C, Acosta EP. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. AIDS 2004, 18:2391-2400.
15. Pushnakit T, Tanpanboon P, Auripul L, Cressley TR, Sirisanthana V. Plasma efavirenz concentrations and the association with CYP2B6*516G>T polymorphism in HIV-infected Thai children. Antivir Ther 2009, 14:315-320.
16. Chantaramisu S, Cressey TR, Maharajmongsiri K, Capparelli E, Tawon Y, Ngo-Giang-Huong N, Jourdain G, Lallement M, Charantita W. Influence of CYP2B6 polymorphisms on the persistence of plasma nevirapine concentrations following a single in-patient dose for the prevention of mother to child transmission in HIV-infected Thai women. J Antimicrob Chemother 2009.
17. Manosuthi W, Sattawawatwong P, Sanaseena L, Koopmans PP, Hekster YA. Determination of nevirapine, an HIV-1 non-nucleoside reverse transcriptase inhibitor, in human plasma by reversed-
phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 2000, 744:65-71.

26. Ramachandran G, Hemanth Kumark AK, Rajasekaran S, Kumar P, Ramesh K, Anitha S, Narendran G, Menon P, Gomathi C, Swaminathan S. CYP2B6 G516T polymorphism but not rifampin coadministration influences steady-state pharmacokinetics of efavirenz in human immunodeficiency virus-infected patients in South India. Antimicrob Agents Chemother 2009, 53:863-868.

27. Ramachandran G, Ramesh K, Hemanth Kumark AK, Jagan I, Vasantha M, Padmapriyadarsini C, Narendran G, Rajasekaran S, Swaminathan S. Association of high T allele frequency of CYP2B6 G516T polymorphism but not rifampin coadministration influences steady-state pharmacokinetics of efavirenz in human immunodeficiency virus-infected patients. J Antimicrob Chemother 2009, 63:841-843.

28. Kivara A, Lartey M, Sagoe KW, Xexemeku F, Kenu E, Olver-Commey J, Boima V, Sagoe A, Boamah I, Greenblatt DJ, Court MH. Pharmacokinetics of efavirenz when co-administered with rifampin in TB/HIV co-infected patients: pharmacogenetic effect of CYP2B6 variation. J Clin Pharmacol 2008, 48:1032-1040.

29. Wang J, Sonnerborg A, Rane A, Josephson F, Lundgren S, Stahle L, Ingelman-Sundberg M. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. Pharmacogenet Genomics 2006, 16:191-198.

30. Rotger M, Tegude H, Colombo S, Cavassini M, Furrer H, Decosterd L, Blievernicht J, Saussele T, Gunthard HF, Schwab M, Eichelbaum M, Telenti A, Zanger UM. Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. Clin Pharmacol Ther 2007, 81:557-566.

31. Manosuthi W, Sungkanuparph S, Tantanathip P, Mankatitham W, Lueangniyomkul A, Thongyen S, Eampokarap B, Uttayamakul S, Suwanvattana P, Kanwai Sad, Rurungham K. Body weight cutoff for daily dosage of efavirenz and 60-week efficacy of efavirenz-based regimen in human immunodeficiency virus and tuberculosis coinfected patients receiving rifampin. Antimicrob Agents Chemother 2009, 53:4545-4548.

32. Kivara A, Lartey M, Sagoe KW, Kenu E, Court MH. CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. AIDS 2009, 23:2101-2106.

33. Wyen C, Hendra H, Vogel M, Hoffmann C, Knechten H, Brockmeyer NH, Bogner JR, Rockstroh J, Esser S, Jaeger H, Harrer T, Mauss S, van Lunzen J, Skoetz N, Nett W, Godecuer C, Faktenheuer G, Kho Si, Egan D, Back DJ, Owen A. Impact of CYP2B6 983T>C polymorphism on non-nucleoside reverse transcriptase inhibitor plasma concentrations in HIV-infected patients. J Antimicrob Chemother 2008, 61:914-918.

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