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Short communication

CYP2B6 haplotype and biological factors responsible for hepatotoxicity in HIV-infected patients receiving efavirenz-based antiretroviral therapy☆

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ABSTRACT

Data on the pharmacogenetic markers of *CYP2B6* and biological factors associated with hepatotoxicity in HIV-infected patients receiving an efavirenz-based antiretroviral therapy (ART) regimen are very limited. A total of 134 HIV-infected Thai adults were prospectively enrolled to receive a once-daily regimen of efavirenz 600 mg/tenofovir/lamivudine. Seven single nucleotide polymorphisms (SNPs) within *CYP2B6* were genotyped using real-time PCR. At 12 weeks after ART, plasma efavirenz concentrations at 12 h after dosing were measured. The mean ± standard deviation patient age was 37 ± 8 years, and 77.6% were male. The median (IQR) CD4 count was 43 cells/mm^3 (17–105 cells/mm^3). Eighteen patients (13.4%) had positive anti-HCV and 5 patients (3.7%) had positive HBsAg. The frequencies of heterozygous/homozygous mutants of each SNP were 64C>T (11%), 499C>G (0%), 516G>T (55%), 785A>G (63%), 1375A>G (0%), 1459C>T (3%) and 21563C>T (62%). The three most frequent haplotypes identified included *1/*6 (40.3%), *1/*1 (34.3%) and *6/*6 (8.2%). The median (IQR) plasma efavirenz concentration was 2.3 mg/L (1.4–3.7 mg/L). At 24 weeks, median (IQR) serum ALP was 98 mg/dL (73–133 mg/dL) and direct bilirubin was 0.11 mg/dL (0.10–0.19 mg/dL). The proportion of grade 1 and grade 2 elevated serum ALP was 12.7% and 1.5%, respectively. By multivariate analysis, factors associated with high ALP, total bilirubin and direct bilirubin included *CYP2B6* haplotype *6/*6, high serum ALP at Week 0 and positive anti-HCV (all $P < 0.05$). In summary, HIV-infected patients with the pharmacogenetic marker '*CYP2B6* haplotype *6/*6' may have increased susceptibility to hepatotoxicity with efavirenz-based ART.

1. Introduction

Efavirenz (EFV) is metabolised via hepatic cytochrome P450 and is both a substrate for and inducer of the 2B6 and 3A4 isoforms of the cytochrome P450 system [1,2]. Variable activity of cytochrome P450 results in interpatient variability in drug clearance, efficacy and toxicity. To date, the range of recommended plasma concentrations of EFV at 12 h after dosing is proposed to be 1–4 mg/L [3]. With regard to pharmacogenetic factors and toxicity, previous studies have shown the relationship among *CYP2B6* polymorphisms, high plasma EFV concentrations and neuropsychiatric adverse events [4]. Data regarding *CYP2B6* haplotypes incorporated with plasma EFV concentrations and biological factors related to EFV-associated hepatotoxicity are very scanty to date. There have been only a few studies in human immunodeficiency virus (HIV)-infected patients revealing an association between *CYP2B6* and hepatotoxicity [5,6]. In addition, the frequency of *CYP2B6* mutant alleles and the incidence of hepatotoxicity varies among ethnic groups. Therefore, this study was conducted to examine the pharmacogenetic markers of *CYP2B6*, plasma EFV concentrations and biological factors associated with hepatotoxicity in HIV and tuberculosis (TB) co-infected patients who are vulnerable to hepatotoxicity.

2. Materials and methods

This study was a prospective, open-label trial involving 156 adult Thai patients co-infected with HIV and TB in order to study

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Table 1
Clinical characteristics and baseline laboratory parameters of 134 HIV/TB co-infected patients.

Characteristic	
Demographics	
Male sex [*n* (%)]	104 (77.6)
Age (years) (mean ± S.D.)	37 ± 8
Body weight (kg) (mean ± S.D.)	54 ± 11
Sites of TB [*n* (%)]	
Lung	63 (47.0)
Cervical lymph node	12 (9.0)
Disseminated TB	54 (40.3)
Meninges	3 (2.2)
Colon	2 (1.5)
Time from starting TB treatment to ART initiation (months) [median (IQR)]	2.1 (1.0–2.8)
Duration of TB treatment (months) [median (IQR)]	8.4 (6.5–9.7)
Received rifampicin-containing antituberculous regimen [*n* (%)]	99 (73.9)
Baseline laboratory parameters	
CD4 cell count (cells/mm^3) [median (IQR)]	43 (17–105)
Percentage CD4 cells (%) [median (IQR)]	6 (3–11)
Plasma HIV-1 RNA (log copies/mL) [median (IQR)]	5.8 (5.4–6.3)
Haemoglobin (g/dL) [median (IQR)]	10.9 (9.7–12.0)
Serum ALP (mg/dL) [median (IQR)]	101 (73–168)
Abnormalities [*n* (%)]	
Grade 1	33 (24.6)
Grade 2	11 (8.2)
Grade 3	2 (1.5)
AST (U/L) [median (IQR)]	38 (27–52)
Abnormalities [*n* (%)]	
Grade 1	30 (22.4)
Grade 2	12 (9.0)
ALT (U/L) [median (IQR)]	31 (18–46)
Abnormalities [*n* (%)]	
Grade 1	23 (17.2)
Grade 2	3 (2.2)
Total bilirubin (mg/dL) [median (IQR)]	0.41 (0.30–0.71)
Grade 1 [*n* (%)]	16 (11.9)
Direct bilirubin (mg/dL) [median (IQR)]	0.2 (0.1–0.4)
Grade 1 [*n* (%)]	9 (6.7)
Grade 2 [*n* (%)]	17 (12.7)
Grade 3 [*n* (%)]	8 (6.0)
Grade 4 [*n* (%)]	3 (2.2)
Albumin (mg/dL) [median (IQR)]	3.4 (3.1–3.8)
Globulin (mg/dL) [median (IQR)]	4.8 (4.2–5.6)
Albumin/globulin ratio [median (IQR)]	0.7 (0.6–0.8)
Total cholesterol (mg/dL) [median (IQR)]	179 (153–205)
Serum creatinine (mg/dL) [median (IQR)]	0.7 (0.6–0.8)
HBsAg-positive	5 (3.7)
HCV antibody positive	18 (13.4)
Pharmacogenetic parameters	
CYP2B6 SNPs: percentage of wild-type/heterozygous mutant/homozygous mutant (%)	
64C>T	89/10/1
499C>G	100/0/0
516G>T	45/47/8
785A>G	37/53/10
1375A>G	100/0/0
1459C>T	97/3/0
21563C>T	38/57/5
CYP2BC haplotypes (%)	
*1/*1	34.3
*1/*2	7.5
*1/*4	2.2
*1/*6	40.3
*2/*4	1.5
*2/*6	1.5
*4/*6	3.0
*5/*6	1.5
*6/*6	8.2

HIV, human immunodeficiency virus; TB, tuberculosis; S.D., standard deviation; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus; SNP, single nucleotide polymorphism.

the frequency of *CYP2B6* single nucleotide polymorphisms (SNPs) and haplotypes at Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand. The study was approved by the institutional ethics committees of Bamrasnaradura Infectious Diseases Institute and the Thai Ministry of Public Health. The period of enrolment was between October 2009 and May 2011. All patients provided written informed consent prior to enrolment. Patients were followed until 24 weeks after initiation of antiretroviral therapy (ART) to examine pharmacogenetic markers of *CYP2B6*, plasma EFV concentrations and biological factors associated with hepatotoxicity.

The initial inclusion criteria included: (i) HIV-infected patients aged 18–60 years; (ii) newly clinically diagnosed active TB, positive acid-fast staining or a positive culture for *Mycobacterium tuberculosis*; (iii) treated with an antituberculous regimen 4–12 weeks prior to enrolment; (iv) naïve to ART; (v) baseline CD4 count < 350 cells/mm^3; and (vi) participated and provided informed consent. Exclusion criteria were as follows: (i) serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 5 times the upper limit of the normal range (ULN); (ii) serum creatinine > 2 times ULN; (iii) receiving immunosuppressive drugs; and (iv) being pregnant or lactating.

All patients were started on a once-daily ART regimen of EFV 600 mg combined with tenofovir 300 mg and lamivudine 300 mg at bed time. ART was initiated between 4 weeks and 12 weeks after initiation of TB treatment. The standard antituberculous regimen included isoniazid, rifampicin, pyrazinamide and ethambutol for the first 2 months followed by isoniazid and rifampicin for the subsequent 4–7 months. Patients who received other antituberculous regimens without rifampicin were those who initially could not tolerate rifampicin owing to adverse effects or hypersensitivity prior to enrolment. Patients had follow-up visits at Weeks 2, 6, 12 and 24 after initiation of ART. Hepatotoxicity was defined based on the AIDS Clinical Trial Group (ACTG) using levels of ALT, AST and ALP to assess severity, with the values expressed as multiples of the ULN: grade 0, <1.25; grade 1, 1.25–2.5; grade 2, >2.5–5; grade 3, >5–10; and grade 4, >10. Increasing bilirubin levels were classified as follows (multiples of the ULN): grade 1, >1.0–1.5; grade 2, >1.5–2.5; grade 3, >2.5–5.0; or grade 4, >5.0. The ULN values for serum ALP, AST, ALT, total bilirubin and direct bilirubin were 129 U/L, 37 U/L, 41 U/L, 1.0 mg/dL and 0.3 mg/dL, respectively.

At baseline, DNA was isolated from stored ethylene diamine tetra-acetic acid (EDTA)-blood cell pellets using a QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany). Genomic DNA was quantified using an ND-1000 ultraviolet spectrophotometer at 260 nm (NanoDrop Technologies, Wilmington, DE). A total of seven SNPs within *CYP2B6* were genotyped. SNPs 516G>T and 785A>G have previously been reported to influence EFV concentrations [7], and *CYP2B6* SNP 21563C>T was identified using the International HapMap Project (http://www.hapmap.org) on Japanese and Han Chinese. SNP 499C>G was associated with high plasma EFV concentrations in Japanese, and the remaining three SNPs were reported in Chinese, i.e. 64C>T, 1375A>G and 1459C>T [8]. *CYP2B6* haplotype determination was interpreted using The Human Cytochrome P450 Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2b6.htm). All SNPs were included for *CYP2B6* haplotype interpretation. Haplotype *1/*1 was defined as no heterozygous and homozygous mutant of seven SNPs.

The plasma EFV concentration at 12 h after dosing was measured using a validated high-performance liquid chromatography (HPLC) assay at 12 weeks after ART initiation while receiving antituberculous treatment. This assay was developed at the Department of Clinical Pharmacology at the University Medical Centre Nijmegen (Nijmegen, The Netherlands). Plasma EFV concentrations were measured at the HIV Netherlands–Australia–Thailand (HIV-NAT) Research Collaboration, Thai Red Cross AIDS Research Centre.

CD4 count by flow cytometry, plasma HIV-1 RNA by real-time PCR, and liver chemistry were assessed at Weeks 0 and 24 after ART.

Frequencies (%) and median [interquartile range (IQR)] were used to describe clinical and laboratory parameters. All possible risk factors associated with hepatotoxicity were evaluated with a linear regression model by adjusting for confounding factors. A *P*-value of <0.05 was considered statistically significant. Independent parameters that were strongly correlated with each other were not analysed in the same multivariate analysis model. All analyses were performed using SPSS v.15.0 (SPSS Inc., Chicago, IL).

3. Results

Of 156 enrolled patients, 22 patients prematurely discontinued EFV before 24 weeks. Reasons for discontinuation included 8 deaths, 5 EFV-associated skin rashes, 4 lost to follow-up, 4 antiretroviral resistances and 1 transfer-out; none of these patients had experienced EFV-associated hepatotoxicity. Thus, 134 HIV-infected Thai adult patients were included in the analysis. Patients' clinical characteristics and baseline laboratory parameters are shown in Table 1. The median (IQR) plasma EFV concentration was 2.3 mg/L (1.4–3.7 mg/L); 7.2 mg/L (5.0–10.1 mg/L) in those with haplotype *6/*6 and 2.1 mg/L (1.4–3.4 mg/L) in those with non-*6/*6 haplotype ($P < 0.001$). Overall, interpatient variability of EFV concentrations was 96%. At 24 weeks, the median (IQR) serum ALP was 98 mg/dL (73–133 mg/dL), AST was 31 U/L (26–46 U/L), ALT was 30 U/L (23–46 U/L), total bilirubin was 0.33 mg/dL (0.25–0.47 mg/dL) and direct bilirubin was 0.11 mg/dL (0.10–0.19 mg/dL). The proportions of patients with grades 1, 2 and 3 abnormality, respectively, were 12.7%, 1.5% and 0% for serum ALP, 17.2%, 3.7% and 0% for AST and 13.5%, 3.7% and 0% for ALT. The proportion of patients with grades 1, 2 and 3 abnormality, respectively, were 1.5%, 0.7% and 0% for total bilirubin and 1.5%, 0% and 2.2% for direct bilirubin. None of the patients had grade 4 abnormalities.

Univariate and multivariate analyses of possible factors associated with high serum ALP level and direct bilirubin are shown in Tables 2 and 3, respectively. By multivariate analysis, predictive factors associated with high serum ALP included haplotype *6/*6, high baseline serum ALP and positive anti-hepatitis C virus (anti-HCV) antibody ($P < 0.05$). The predictive factors associated with high direct bilirubin included haplotype *6/*6, high baseline serum ALP and positive anti-HCV antibody ($P < 0.05$). A similar trend was found in the model of total bilirubin as a dependent variable ($P < 0.05$). There was no relationship between high transaminase enzymes, including AST and ALT, and all study predictive parameters ($P > 0.05$).

4. Discussion

To date, the utility of a pharmacogenetic marker to predict the likelihood of EFV-associated hepatotoxicity is very limited. *CYP2B6* is genetically polymorphic [9,10], thus determination of haplotype would be a better approach than a SNP. This is the first study that has shown a strong correlation between a predictive pharmacogenetic factor and hepatic cholestasis in HIV/TB co-infected patients who had received EFV-based ART. A recent previous study by Yimer et al. in HIV-monoinfected Ethiopians revealed an association between *CYP2B6* *6/*6 as a putative genetic marker and liver injury [5].

Although no patient had symptomatic hepatitis, high levels of all key biological markers of hepatic cholestasis, including serum ALP, total bilirubin and direct bilirubin, were significantly associated with *CYP2B6* haplotype *6/*6. Different polymorphisms in the particular genes influence the expression of CYP2B6 that contributes to the variation in EFV toxicity [4]. In addition, EFV is mainly metabolised via CYP2B6, and to a lesser extent via other pathways, e.g. CYP3A4 and CYP2A6 [1,2,11]. Of note, hepatic cholestasis was a predominant abnormal hepatic profile found in this study. Rifampicin co-administration may play a role owing to rifampicin itself causing cholestasis, particularly in HIV-infected patients [12]. Nevertheless, the parameter 'receiving rifampicin' was not found to be significantly associated with hepatotoxicity, because most of the study patients had received a rifampicin-containing antituberculous regimen. However, antiretroviral-drug-associated hepatotoxicity manifests as either hepatocellular injury or hepatic cholestasis. On the other hand, markers of hepatocellular injury were not found to be associated with *CYP2B6* haplotype *6/*6 in the present study. A previous study has shown a correlation between this haplotype and transaminitis in Africans [5]. Most of the patients in this cohort had mild hepatotoxicity, either cholestasis or transaminitis without clinical symptoms. This finding may be explained by the fact that the present study enrolled patients who had relatively normal liver chemistry without advanced stage of liver disease and they had all been clinically stable with EFV-based ART for a period of time. ART initiation while receiving TB treatment was not consistent among studies, thus comparison of their data may not be feasible. However, this finding should raise a clinical concern in the treatment of patients with chronic liver disease who receive EFV-based ART. Further studies are needed to confirm the clinical utility in such patients.

One of the proposed mechanisms of non-nucleoside reverse transcriptase inhibitor-associated hepatotoxicity is dose-dependent [13]. The observed high interpatient variability of plasma EFV concentrations may play a role regarding hepatotoxicity. However, there appears to be no correlation between high plasma EFV concentration and hepatotoxicity in the multivariate analysis. A previous study showed an association between high EFV concentration as an intermediate marker and transaminitis [5]. The different frequencies of *CYP2B6* mutant alleles between ethnic groups, the relatively small sample size and other unknown mechanisms of EFV-induced hepatotoxicity may be explanations for this unrelated intermediate marker. In addition, the peak time point of EFV-induced hepatotoxicity is within the first few weeks after ART initiation. The two time points for liver chemistry measurements in the present study were at Weeks 0 and 24. EFV is a cytochrome P450 enzyme inducer, resulting in the induction of its own metabolism. Given this fact, plasma EFV measurements were performed after the peak incidence of the transient period of hepatotoxicity. This may be another reason why the association of EFV concentration and hepatotoxicity was not found. Thus, the factor 'high EFV concentration' plays a role in hepatotoxic involvement.

Another observation is that HCV co-infection is a significant factor predicting hepatotoxicity. This finding is similar to other antiretroviral regimens in patients who were co-infected with HIV and HCV including protease inhibitor-based ART [14]. HCV co-infection was an important determining factor, increasing two- to seven-fold the risk of hepatotoxicity [13]. The severity of liver injury before and after initiating ART plays a role. The potential for hepatotoxicity may be exacerbated by this co-infection, especially in those patients who carry *CYP2B6* haplotype *6/*6. On the other hand, hepatitis B virus (HBV) co-infection was not found to be associated with hepatotoxicity. This finding can be explained by the fact that tenofovir and lamivudine were a component of the backbone nucleoside reverse transcriptase inhibitors used in the present study. They have antiviral activities both against HIV and HBV. In addition, the proportion of patients who were co-infected with HBV was relatively small (3.7%). Given sex-dependent hepatotoxicity that is correlated with

Table 2
Univariate and multivariate analyses of high serum alkaline phosphatase (ALP) level at Week 24 as the dependent variable (treated as a continuous variable).

Parameter	Univariate analysis		Multivariate analysis			
	P-value	β	Model I[a]		Model II	
			P-value	β	*P*-value	β
Body weight at Week 0	0.150	−0.865	–	–	–	–
Male sex	0.271	16.830	–	–	–	–
Age	0.936	0.062	–	–	–	–
Extrapulmonary/disseminated TB	0.120	19.799	–	–	–	–
Receiving rifampicin-containing regimen	0.246	−0.099	–	–	–	–
Serum ALP at Week 0	<0.001	0.310	<0.001	0.276	<0.001	0.288
AST at Week 0	0.427	0.167	–	–	–	–
ALT at Week 0	0.610	−0.127	–	–	–	–
Total bilirubin at Week 0	0.046	30.878	0.704	4.822	0.714	4.722
Direct bilirubin at Week 0	0.025	36.588	–	–	–	–
HBsAg-positive	0.537	−20.771	–	–	–	–
HCV antibody positive	0.001	61.024	0.026	34.408	0.019	36.748
Percentage CD4 cells	0.042	1.845	0.255	0.837	0.359	0.689
Log plasma HIV-1 RNA	0.256	11.499	–	–	–	–
Total cholesterol at Week 0	0.956	0.009	–	–	–	–
Efavirenz concentration	0.088	3.475	–	–	0.129	2.582
CYP2B6 haplotypes						
*1/*1	0.515	−8.746	–	–	–	–
*1/*2	0.327	−23.769	–	–	–	–
*1/*4	0.709	−16.089	–	–	–	–
*1/*6	0.702	−4.974	–	–	–	–
*2/*4	0.968	2.136	–	–	–	–
*2/*6	0.815	12.288	–	–	–	–
*4/*6	0.744	−12.262	–	–	–	–
*5/*6	0.887	−7.508	–	–	–	–
*6/*6	0.002	71.868	0.019	44.666	–	–

TB, tuberculosis; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus.
[a] Efavirenz concentration and direct bilirubin were not included in the multivariate analysis model I because of strong correlations between efavirenz concentration and haplotype *6/*6, and total bilirubin and direct bilirubin, respectively.

nevirapine [13], female sex was not found to be associated with hepatotoxicity.

A number of limitations need to be addressed. First, EFV-related hepatitis generally occurred within the first few weeks. This event might therefore compromise the relevance of the haplotype and hepatotoxicity association analysis. However, all baseline clinical characteristics and *CYP2B6* haplotypes were not different between 22 patients who prematurely discontinued the study drug and the

Table 3
Univariate and multivariate analyses of high direct bilirubin level at Week 24 as the dependent variable (treated as a continuous variable).

Parameter	Univariate analysis		Multivariate analysis			
	P-value	β	Model I[a]		Model II	
			P-value	β	*P*-value	β
Body weight at Week 0	0.550	0.052	–	–	–	–
Male sex	0.214	−0.108	–	–	–	–
Age	0.988	0.001	–	–	–	–
Extrapulmonary/disseminated TB	0.459	0.064	–	–	–	–
Receiving rifampicin-containing regimen	0.815	0.020	–	–	–	–
Serum ALP at Week 0	0.001	0.286	0.007	0.216	0.003	0.248
AST at Week 0	0.648	−0.040	–	–	–	–
ALT at Week 0	0.797	−0.023	–	–	–	–
Total bilirubin at Week 0	0.149	0.125	–	–	–	–
Direct bilirubin at Week 0	0.116	0.136	–	–	–	–
HBsAg-positive	0.861	−0.015	–	–	–	–
HCV antibody positive	<0.001	0.307	0.007	0.218	0.003	0.250
Percentage CD4 cells	0.619	0.043	–	–	–	–
Log plasma HIV-1 RNA	0.702	0.033	–	–	–	–
Total cholesterol at Week 0	0.339	0.083	–	–	–	–
Efavirenz concentration	0.094	0.145	–	–	0.222	0.100
CYP2B6 haplotypes						
*1/*1	0.283	−0.093	–	–	–	–
*1/*2	0.648	−0.040	–	–	–	–
*1/*4	0.695	−0.034	–	–	–	–
*1/*6	0.464	−0.064	–	–	–	–
*2/*4	0.811	−0.021	–	–	–	–
*2/*6	0.783	−0.024	–	–	–	–
*4/*6	0.938	−0.007	–	–	–	–
*5/*6	0.867	0.015	–	–	–	–
*6/*6	<0.001	0.488	0.001	0.278	–	–

TB, tuberculosis; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBsAg, hepatitis B virus surface antigen; HCV, hepatitis C virus.
[a] Efavirenz concentration was not included in the multivariate analysis model I because of a strong correlation between efavirenz concentration and haplotype *6/*6.

remaining 134 patients who completed follow-up ($P > 0.05$, data not shown). Second, other possible causes of cholestatic disease were not completely excluded, including infiltrative liver disease, biliary tract disease and concurrent opportunistic infections. Careful history review and physical examination of patients had been done. Nevertheless, none of the patients subsequently developed clinical significance related to such diseases. Third, the γ-glutamyl transpeptidase level was not determined. However, elevations of other hepatic cholestasis markers in this study were indicative of hepatic cholestasis. Fourth, PCR of HCV was not performed. Some of the patients with positive anti-HCV might have negative PCR results owing to viral clearance. Nonetheless, HCV co-infection is found to be a significant factor to predict hepatotoxicity in this study and in previous reports. Ultimately, the majority of patients had only a mild degree of hepatotoxicity. Thus, a further larger-scale study is needed to explore the clinical relevance of this finding.

The present study provides interesting data with regard to the predictive factors contributing to hepatotoxicity in HIV-infected Thai patients. Patients who have the pharmacogenetic marker '*CYP2B6* haplotype *6/*6' had markedly increased susceptibility to hepatotoxicity with an EFV-based ART regimen. In addition, patients who have co-infection with HCV and high baseline serum ALP are also vulnerable to hepatotoxicity. Taken together, EFV-associated hepatotoxic vulnerability reflects the combined influence of a pharmacogenetic factor and biological factors, but in the different magnitudes.

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Competing interests: None declared.

Ethical approval: The study was approved by the institutional ethics committees of Bamrasnaradura Infectious Diseases Institute (Nonthaburi, Thailand) and the Thai Ministry of Public Health.

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