รายงานวิจัยฉบับสมบูรณ์

การศึกษาเภสัชพันธุศาสตร์และการแปลผล haplotype ของยีน CYP 2B6 ซึ่งมีความสัมพันธ์กับการออกฤทธิ์ของยาเอฟาวิเรนซ์ในผู้ติดเชื้อเอชไอวีแบบที่ 1

Pharmacogenetics study and haplotype determination of CYP2B6 correlated with plasma efavirenz concentration in HIV-1 infection

โดย ผู้ช่วยศาสตราจารย์ เภสัชกร ดร. ชลภัทร สุขเกษม

มิถุนายน 2556

สัญญาเลขที่สัญญาMRG5480136

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย
(ความเห็นในรายงานนี้เป็นของผู้วิจัย
สกว. ไม่จำเป็นต้องเห็นด้วยเสมอไป)

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เอกสารแนบหมายเลข 2

Abstract (บทคัดย่อ)

Project Code : MRG5480136
(รหัสโครงการ)

Project Title : การศึกษาเภสัชพันธุศาสตร์และการแปลผล haplotype ของยีน CYP 2B6
(ชื่อโครงการ) ซึ่งมีความสัมพันธ์กับการออกฤทธิ์ของยาเอฟาวิเรนซ์ในผู้ติดเชื้อเอชไอวี แบบที่ 1
Pharmacogenetics study and haplotype determination of CYP2B6 correlated with plasma efavirenz concentration in HIV-1 infection

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Project Period : 2 ปี (15 มิถุนายน 2554 ถึง 15 มิถุนายน 2556)
(ระยะเวลาโครงการ)

CYP2B6 ควบคุมการสร้างเอนไซม์ CYP 2B6 ซึ่งมีบทบาทสำคัญในการเมทาบอไลซ์ของยาเอฟาวิเรนซ์ ปัจจุบันยังไม่มีการศึกษาถึงความหลากหลายทางพันธุกรรม (polymorphism) ของยีน CYP2B6 และความสัมพันธ์ระหว่างความหลากหลายทางพันธุกรรมของยีน CYP2B6 กับระดับยาเอฟาวิเรนซ์ในกระแสเลือดกลุ่มผู้ติดเชื้อเอชไอวี และกลุ่มผู้ติดเชื้อเอชไอวีร่วมกับเชื้อวัณโรคในประเทศไทย การศึกษาวิจัยนี้ได้ดำเนินการศึกษาความหลากหลายทางพันธุกรรมของยีน CYP2B6 แบบการเปลี่ยนแปลงเบสหนึ่งเบส (single nucleotide polymorphisms: SNP) 9 ตำแหน่ง ร่วมกับการวิเคราะห์ระดับยาเอฟาวิเรนซ์ เพื่อหาความสัมพันธ์ระหว่างความหลากหลายทางพันธุกรรมของยีน CYP2B6 กับระดับยาเอฟาวิเรนซ์ในกระแสเลือด พบว่าผู้ป่วยที่มี SNP c.516G>T, c.785A>G และ g.21563C>T จะมีระดับยาเอฟาวิเรนซ์ในกระแสเลือดสูงกว่าคนทั่วไป และเกินกว่าระดับของ

การรักษา (Therapeutic level คือ 1,000-4000 ng/ml) ซึ่งอาจส่งผลให้ผู้ป่วยเกิดภาวะความเป็นพิษต่อระบบประสาท (CNS toxicity) ได้ ทั้งนี้พบว่าคนไทยมีการเกิดความผิดแผกทางพันธุกรรมของทั้ง 3 SNP (c.516G>T, c.785A>G และ g.21563C>T) ในอัตราร้อยละ 36%, 41% และ 35% ตามลำดับ ซึ่งแสดงถึงความเสี่ยงต่อการเกิดอาการไม่พึงประสงค์ในคนไทยซึ่งเป็นผู้ติดเชื้อเอชไอวีกรณีต้องได้รับการรักษาด้วยยาเอฟาวิเรนซ์ในขนาดมาตรฐาน (600 มก.ต่อวัน) นอกจากนี้ยังพบว่ากลุ่มตัวอย่างที่มีความผิดแผกทางพันธุกรรมที่ตำแหน่ง g.18492T>C มีแนวโน้มต่อการเกิดระดับยาอีฟาวิเรนซ์ในกระแสเลือดต่ำกว่าการรักษาได้ (Sub-optimal level) ซึ่งอาจส่งผลให้เกิดการกลายพันธ์ของเชื้อเอชไอวีซึ่งสัมพันธ์ต่อการเกิดภาวะดื้อยาได้ง่าย ทั้งในกลุ่มผู้ติดเชื้อเอชไอวี และกลุ่มผู้ติดเชื้อเอชไอวีร่วมกับเชื้อวัณโรคในประเทศไทย

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) which is mainly metabolized by hepatic cytochrome P450 2B6 (CYP2B6). The preferable mid-dosing plasma level of efavirenz is 1,000–4,000 ng/mL to allow for optimized antiretroviral potency and to minimize the risk for neuropsychiatric toxicity. This study aimed to examine the frequencies of CYP2B6 and the association between CYP2B6 polymorphisms and plasma efavirenz concentrations in HIV-1 infected patients and HIV/Tuberculosis Co-Infected Patients. Mid-dose plasma efavirenz concentration was determined following the initiation of an antiretroviral therapy (tenofovir, lamivudine and efavirenz) using HPLC/MS/MS. Candidate CYP2B6 polymorphisms were conducted by real-time PCR-based allelic discrimination. Our studies of CYP2B6 polymorphisms showed significant allelic variants (CYP2B6 c.516G>T and c.785A>G polymorphisms) which may decrease the clearance of efavirenz by reducing the activity of CYP2B6 enzyme and thereby increase plasma efavirenz concentration in HIV-1 infected patients and HIV/Tuberculosis Co-Infected Patients. Moreover, the median efavirenz concentration for patients with the g.18492 heterozygous variants or homozygous variants was significantly lower than those with the wild-type genotype. The information given by this single-SNP analysis may help to effectively identify HIV-infected individuals who may have a risk for treatment failure. Because the T allele in CYP2B6 g.18492C>T have high frequencies among HIV-infected population. Therefore, the impact of SNPs, which are correlated with high dose of efavirenz plasma concentrations, was found. The genetic configuration of SNPs, which are associated with plasma efavirenz levels, may be useful in optimizing the efavirenz dose that is used in HIV-1 infected patients.

Keywords: CYP2B6, Efavirenz, Polymorphism, Pharmacogenetics, SNP, Plasma drug concentration

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Executive Summary

ยีน CYP2B6 อยู่บนโครโมโซมคู่ที่ 19 (19q13.2) ทำหน้าที่สร้างเป็นเอนไซม์ CYP 2B6 ที่มีบทบาทในการย่อยสลายยาอีฟาวิเรนซ์ โดยพบความผิดแผกทางพันธุกรรมในหลายรูปแบบ เช่น CYP2B6*1 (516G/785A/1459C), CYP2B6*2 (C64T), CYP2B6 *3 (C777A), CYP2B6*4 (785G), CYP2B6*5 (1459T), CYP2B6*6 (516T/785G) และ CYP2B6*7 (516T/785G/1459T) โดยผู้ป่วยที่มีความผิดแผกทางพันธุกรรมแบบ CYP2B6*6, CYP2B6*16 และ CYP2B6*18 จะมีความสามารถในการย่อยสลายยาได้น้อยลง การวิจัยครั้งนี้เป็นการศึกษาถึงความหลากหลายทางพันธุกรรมของยีน CYP2B6 ร่วมกับการวิเคราะห์ระดับยาเอฟาวิเรนซ์ซึ่งเป็นยาต้านไวรัสเอชไอวีสูตรพื้นฐานของประเทศไทย (กรณีผู้ป่วยแพ้ยาเนวิราปีน) ในกลุ่มผู้ติดเชื้อเอชไอวี และกลุ่มผู้ติดเชื้อเอชไอวีร่วมกับเชื้อวัณโรคในประเทศไทย จำนวน 202 ราย จากโครงการ PHPT-2 คณะเทคนิคการแพทย์ มหาวิทยาลัยเชียงใหม่ และจากโรงพยาบาลบำราศนราดูร กรมควบคุมโรค กระทรวงสาธารณสุข

จากการศึกษาดังกล่าว พบความสัมพันธ์ระหว่างความหลากหลายทางพันธุกรรมของยีน CYP2B6 กับระดับยาเอฟาวิเรนซ์ในกระแสเลือดของผู้ติดเชื้อเอชไอวีที่เป็นกลุ่มประชากรไทย คือ ความผิดแผกของ 3 SNP (c.516G>T, c.785A>G และ g.21563C>T) จะส่งผลต่อระดับยาเอฟาวิเรนซ์ โดยทำให้ระดับยาในกระแสเลือกของผู้ป่วยสูงมากขึ้น และเกินกว่าระดับของการรักษา (Therapeutic level คือ 1,000-4000 ng/ml) ซึ่งอาจส่งผลให้ผู้ป่วยเกิดอาการไม่พึงประสงค์จากการใช้ยา เช่น ภาวะความเป็นพิษต่อระบบประสาท (CNS toxicity) ทั้งนี้ความผิดแผกทางพันธุกรรมทั้ง 3 SNP (c.516G>T, c.785A>G และ g.21563C>T) มีโอกาสพบได้มากในกลุ่มประชากรไทยที่เป็นผู้ติดเชื้อเอชไอวี คิดเป็น 0.365, 0.413 และ 0.356 ตามลำดับ ซึ่งแสดงถึงความเสี่ยงต่อการเกิดอาการไม่พึงประสงค์ในกลุ่มประชากรไทยที่เป็นผู้ติดเชื้อเอชไอวี และกลุ่มผู้ติดเชื้อเอชไอวีร่วมกับเชื้อวัณโรคที่มีโอกาสได้รับยาเอฟาวิเรนซ์ ในขนาดมาตรฐานคือ 600 มก.ต่อวัน

นอกจากนี้ยังพบว่าความผิดแผกทางพันธุกรรมที่ตำแหน่ง g.18492T>C แบบ TC, CC (heterozygous and homozygous variant) จะมีระดับยาเอฟาวิเรนซ์ต่ำกว่าแบบ TT (homozygous wild-type) ซึ่งผู้ป่วยอาจมีความเสี่ยงต่อการเกิดระดับยาต่ำกว่าการรักษาได้ ซึ่งพบว่า SNP ดังกล่าวมีการศึกษาในกลุ่มประชากรชาวชิลี แต่ไม่พบความสัมพันธ์ต่อระดับยาอย่างมีนัยสำคัญ ทั้งนี้ยังไม่เคยมีการศึกษาในกลุ่มคนเอเชียเลย ดังนั้นจึงเป็นการศึกษาแรกที่ทำในคนเอเชีย และพบความสัมพันธ์ต่อระดับยาเอฟาวิเรนซ์ที่น่าสนใจ ทั้งนี้เนื่องจากกรณีที่ผู้ป่วยได้รับการรักษาด้วยยาต้านที่มียาเอฟาวิ

เรนซ์เป็นองค์ประกอบในขนาดมาตรฐาน 600 มก.ต่อวัน อาจมีความเสี่ยงต่อการมีระดับยาต่ำกว่าการรักษา (Sub-optimal level) อาจส่งผลให้เกิดการกลายพันธ์ของเชื้อเอชไอวีซึ่งสัมพันธ์ต่อการเกิดภาวะดื้อยาได้ง่าย ที่สำคัญกว่านั้นคือผู้ป่วยอาจมีโอกาสส่งผ่านเชื้อที่มียีนดื้อยาเหลานี้ไปให้กับผู้ติดเชื้อรายใหม่ได้ (treatment naïve infection) ซึ่งผู้ป่วยรายใหม่ก็จะไม่สามารถใช้ยาต้านไวรัสเอชไอวีสูตรพื้นฐานของประเทศไทย (กรณีผู้ป่วยแพ้ยาเนวิราปีน) จำเป็นต้องใช้ยาสูตรอื่นที่แพงขึ้น ทำให้สิ้นเปลืองงบประมาณในการดูแลรักษา และยังส่งผลกระทบในวงกว้างทางการสาธารณสุขของประเทศได้

ดังนั้นการตรวจวินิจฉัยทางเภสัชพันธุศาสตร์ของยีน CYP2B6 จะสามารถใช้ทำนายระดับยาเอฟาวิเรนซ์ในกระแสเลือดของผู้ป่วย เพื่อจะช่วยลดความเสี่ยงในการเกิดอาการไม่พึงประสงค์จากการใช้ยาได้ และการเกิดระดับยาต่ำกว่าการรักษาในกลุ่มประชากรไทยที่เป็นผู้ติดเชื้อได้ ซึ่งจะช่วยให้ผู้ป่วยสามารถรับประทานยาอย่างต่อเนื่องและสม่ำเสมอ ลดโอกาสเกิดการดื้อต่อยาต้านไวรัส ซึ่งทำให้ผู้ป่วยมีชีวิตที่ยืนยาวและมีคุณภาพชีวิตที่ดีขึ้นได้

ผลงานวิจัยที่ทำในรอบ 1 ปี มีดังนี้

(1) งานตีพิมพ์ในวารสารนานาชาติ 7 ฉบับ

(2) งานเขียนหนังสือ 1 บท

(3) Poster presentation ในงานประชุมวิชาการระดับนานาชาติ 3 เรื่อง

(4) Proceeding พร้อมนำเสนอปากเปล่า 1 ครั้งในงานประชุมวิชาการระดับชาติ

(5) การบรรยายในการประชุมวิชาการระดับนานาชาติและระดับชาติ งานประชุมแพทย์ และการฝึกอบรมเชิงปฏิบัติการจำนวนหลายครั้ง

(6) ใช้ประกอบการสอน และเป็นเนื้อหาในเอกสารประกอบการสอนสำหรับนักศึกษาหลักสูตรแพทยศาสตรบัณฑิต นักศึกษาปริญญาโท และปริญญาเอก และการฝึกอบรมแพทย์ประจำบ้าน

(7) มีการนำผลงานวิจัยนี้ไปประยุกต์ใช้ทางคลินิกโดยห้องปฏิบัติการเภสัชพันธุศาสตร์และการแพทย์เฉพาะบุคคล ศูนย์การแพทยสมเด็จพระเทพรัตน์ โรงพยาบาลรามาธิบดี

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OBJECTIVES

1. เพื่อหาความถี่อัลลีนของยีน CYP 2B6 ในตำแหน่งที่มีความผิดแผกทางพันธุกรรมซึ่งมีความสัมพันธ์กับการออกฤทธิ์ของยาเอฟาวิเรนซ์ในกลุ่มประชากรไทย
2. เพื่อให้สามารถแปลงผลการตรวจตำแหน่งที่มีความผิดแผกทางพันธุกรรมเป็นรูปแบบ Haplotype ซึ่งนำไปใช้ในการคำนวณขนาดยาเอฟาวิเรนซ์ในระดับคลินิก
3. ศึกษาความสัมพันธ์ระหว่างตัวบ่งชี้ทางเภสัชพันธุศาสตร์ของยีน CYP 2B6 กับระดับยาเอฟาเรนซ์ในกระแสเลือด
4. เพื่อใช้เป็นข้อมูลในการพัฒนาเทคนิควิธีการตรวจวินิจฉัยตัวบ่งชี้ทางเภสัชพันธุศาสตร์ของยีน CYP 2B6 บนตำแหน่งที่สัมพันธ์กับการออกฤทธิ์ของยาเอฟาวิเรนซ์
5. เพื่อใช้เป็นข้อมูลอ้างอิงในการปรับขนาดยาเอฟาวิเรนซ์ในประชากรไทย ที่มีการตรวจวินิจฉัยตัวบ่งชี้ทางเภสัชพันธุศาสตร์ของยีน CYP 2B6วิธีทดลอง

MATERIALS AND METHODS

1. Sample populations and characteristics

Following written inform consent form, 202 Thai adults with HIV-1 infection and Patients co-infected with HIV and tuberculosis were recruited from PHPT-GFATM treatment program and Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand, respectively. Enrolled patients were aged ≥ 18 years, had no opportunistic infection and were taking antiretroviral regimen with tenofovir (300 mg), lamivudine (300 mg) and efavirenz (600 mg) at bed time. Mid-dose efavirenz plasma concentration was determined at 12 weeks following the initiation of antiretroviral therapy. Patients receiving concomitant treatments that could potentially affect efavirenz pharmacokinetics were excluded. All subjects provided written informed consent within the cohort study, which included the use of stored samples for future research following specific ethical clearance. This study was approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand.

Sample selection criteria

1. Samples from patients will be included if:
2. HIV-infected patient receiving 2 NRTIs plus efavirenz.
3. Plasma sample available after at least 4 weeks of efavirenz treatment and the EFV plasma concentration was determined after consume the last capsule (at standard dose 600 mg) within duration of measure between 12-25 hours.
4. Are receiving no concomitant treatments that affect EFV pharmacokinetics (i.e. CYP450 enzyme induces or inhibitors)
5. Stored cell pellet collected in a EDTA blood collection tube for the extraction of DNA
6. Patient baseline characteristics: age, weight, alanine aminotransferase level (U/L) are available

2. DNA extraction and quantification of patient samples

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Human genomic DNA will be isolated from EDTA cell pellets using the QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany) commercial DNA extraction kit. DNA concentration will be measured using a Nanodrop assay and diluted to 10 ng/ µl.

3. Selection of SNPs within CYP2B6

No SNP database of CYP2B6 in available in the Thai SNP discovery project. To cover a higher proportion of this gene while decreasing the number of SNPs, we selected the candidate SNPs from the representative tag SNPs of each LD by using the SNPs data reported in the Chinese population from the Perlegen Sciences database. If there was more than one SNPs from each LD by the selection was based on functionality of the sequence, i.e. appearing in the exon, promoter and 3'UTR region. The SNPs availabilities in the collection of TaqMan® Assays-on- Demand™ SNP Genotyping Products (Applied Biosystems, CA, and USA) were also taken into account. We also included known 9 SNPs from literature searches in our selection. Primers-Probes for detection of SNPs.

Novel assays using specific primers and fluorescently labeled probes were design to read CYP2B6 (NG_000008). For candidate SNPs unavailable in the collection of TaqMan® "Assays-on-Demand®" SNP Genotyping Products, primers and probes for detection of the SNPs will be designed by TaqMan® "Assays-by-Design®" SNP Genotyping Assay Service (Applied Biosystems, CA, and USA).

4. CYP2B6 Genotyping

A total of six SNPs within CYP2B6 were genotyped (GeneBank accession number NG_000008.7). SNPs c.516G>T (rs3745274) and c.785A>G (rs2279343) have previously been reported to influence enzyme activity and efavirenz concentrations. The c.499C>G (rs3826711) SNP, part of the *26 allele, was associated with high efavirenz plasma concentrations in Japanese subjects, and three other SNPs also assessed in this study, c.64C>T (rs8192709), c.1375A>G (rs3211369), and c.1459C>T (rs3211371) were also selected for assessment.

Pre-designed TaqMan assays (Applied Biosystems, Foster City, CA), were used to genotype the CYP2B6 c.516G>T (assay ID C__7817765_60), c.64C>T (assay ID C__2818162_20), c.499C>G (assay ID C__2752377_10), c.1375A>G (assay ID C__2741754_10), c.1459C>T (assay ID C__30634242_40), g.3003T>C (assay ID C__2818167_10) and g.21563C>T (assay ID C__22275631_10). The CYP2B6 c.785A>G were performed by custom TaqMan assays (Applied Biosystems). The sequences of primers and probes were: CYP2B6 c.785A>G, TGGAGAAGCACCGTGAAACC (forward), TGGAGCAGGTAGGTGTCGAT (reverse), VIC-CCCCCAAGGACCTC-MGB (wild-type), FAM-CCCCAGGGACCTC-MGB (mutant).

For SNP analysis, these 6 SNPs were genotyped using allele-specific fluorogenic 5' nuclease chain reaction assay with predesigned primers and TaqMan MGB probes (TaqMan SNP Genotyping Assay; Applied Biosystems, Foster City, CA). Sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest used two TaqMan MGB probes; one probe was labeled with VIC dye and detected the Allele 1 sequence, the second probe was labeled with FAM dye and detected the Allele 2 sequence.

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3. Sample Preparation and Efavirenz plasma drug concentration Assay

Fasting plasma efavirenz level at 12 hours after dosing was measured using a validated high performance liquid chromatopraphy assay at 12 weeks of ART inititation. Briefly, patient plasma samples (300 µl) and all calibration and control samples were heat inactivated in a water bath at 56 °C for 30 minutes prior to assay. Sample pretreatment involved protein precipitation with acetonitrile (360 µl) and following centrifugation the sample supernatant was injected into the HPLC machine.

Analytic was performed using an Agilent 1100 HPLC machine with an Omnispher C18 (150 x 4.6 mm I.D/particle size 5 µm) analytical column (Varian, CA, USA), a Chromquard RP guard column and a mobile phase consisting of 10 mM KH2PO4 pH 3.1 - acetonitrile (50:50, v/v). The retention time for efavirenz was 5.1 min. The peak of this compound was well resolved and free of interference from endogenous compounds in the plasma. The lower limit of quantification (LLOQ) of efavirenz is 0.2 mg/L. The detection limit was defined as a signal-to-noise ratio of 3:1. The calibration standards for plasma samples were linear in the range of 0.2-20 mg/L. Low, medium and high level QC samples were prepared at 0.4, 1.5 and 10 mg/L, respectively. The intra- and inter-day precision was within 0.0758% and 0.0091%, respectively. The assay accuracy was 93-104%. This assay was developed at the Department of Clinical Pharmacology at the University Medical Centre Nijmegen, The Netherlands. The sample peak heights were processed by ChromQuest Software version 4.1.

4. Statistical analysis

CYP2B6 haplotype determination was interpreted using The Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2b6.htm). All genotype distributions were tested for Hardy-Weinberg equilibrium using exact tests. SNPs with a call rate < 95% were omitted. A Kruskal-Wallis test was used to test for significant difference in efavirenz concentrations between genotypes. Mann-Whitney U tests were used to compare efavirenz concentrations between two genotypes. The QTLHAPLO program was used to identify the association between the CYP2B6 haplotypes and the efavirenz plasma concentration. Statistical significance was defined as $P < 0.05$.

RESULTS

Impact of Pharmacogenetic Markers of CYP2B6 on Efavirenz Levels in HIV/Tuberculosis Co-Infected Patients

One hundred HIV-1 infected patients meeting the inclusion criteria were genotyped for cytochrome P450 2B6 (CYP2B6) polymorphisms for 8 single nucleotide polymorphisms (SNPs; c.64C>T, c.499C>G, c.516G>T, c.785A>G, c.1375A>G and c.1459C>T) and all were in Hardy-Weinberg equilibrium. The minor allele frequency (MAF) for CYP2B6 c.64C>T, c.499C>G, c.516G>T, c.785A>G, c.1375A>G and c.1459C>T was 0.06, 0.00, 0.32, 0.36, 0.00 and 0.015, respectively. Fifty-six patients (56%) had the c.516G>T (homozygous variants), 63 patients (63%) had the c.785A>G

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(homozygous variants). In the entire cohort study, 3 out of 6 SNPs (c.64C>T, c.516G>T and c.785A>G) have statistical significantly different of efavirenz plasma concentration between genotype groups ($P < 0.05$). Subjects who are carriers of variant allele of c.64CT (1.380 mg/L; IQR, 0.903-1.948) and c.64TT genotype (3.200 mg/L; IQR, 3.200-3.200) had significantly different plasma EFV concentration compared with homozygous wild-type form (c.64CC genotype; 2.390 mg/L; IQR, 1.555-3.865) with $P = 0.035$. Median midpoint concentration in c.516TT (7.075 mg/L; IQR, 3.378-8.885) trended higher than those of c.516GT (2.665 mg/L; IQR, 1.945-4.078) and c.516GG (1.570 mg/L; IQR, 1.293-2.520). Moreover, median midpoint concentration in c.785GG (6.940 mg/L, IQR 2.035-8.510) trended higher than those of c.785AG (2.590 mg/L, IQR 1.930-3.853) and c.785AA (1.480 mg/L, IQR 1.265-2.270).

The most frequent CYP2B6 allele in the population tested here was *1 (117/200; 58.5%), and *6 (63/200; 31.5%), whereas only two *5 alleles were identified (1.0%). According to the haplotype, the most frequent haplotypic combinations were *1/*6, *1/*1, *1/*2 and *6/*6 at the frequencies of 42.0%, 32.0%, 8.0% and 7.0%, respectively.

No CYP2B6 polymorphism was detected in 32 patients and their haplotypes were determined to be *1/*1. The haplotypes of single-SNP carriers with c.64CT, c.516GT, c.785AG and c.1459CT were determined to be *1/*2 (n=8), *1/*6 (n=42), *1/*4 (n=3), and *1/*5 (n=0), respectively, whereas those of homozygous carriers with both c.516TT and c.785GG were determined to be *6/*6 (n=7). Two patients with c.64CT, c.516GT, and c.785AG was identified as *2/*6, while 3 patients carrying both c.516GT and c.785GG genotypes but no other polymorphisms, were determined to be *4/*6.

The result of CYP2B6 haplotype associated with efavirenz plasma concentrations are summarized. Among the subjects, the median plasma efavirenz concentration was 2.260 mg/L (IQR, 1.442-3.665) and had a high inter-individual variability ranging from 0.58 mg/L to 23.35 mg/L. Median efavirenz level was 1.570 mg/L (IQR, 1.295-2.670) for individuals homozygous for the CYP2B6 *1/*1 (n = 32/100). From the analysis, significantly elevated of efavirenz levels were found for *1/*6 (n=42/100, $P = 0.0011$) and *6/*6 (n=7/100, $P < 0.00001$) haplotype form. There was no significant difference in the efavirenz levels for carriers of *1/*2, *1/*4, *2/*4, *2/*6, *4/*6 and *5/*6.

Impact of Pharmacogenetic Markers of CYP2B6 on Efavirenz Levels in HIV/Tuberculosis Co-Infected Patients

One hundred and fifty patients were initially enrolled and started ART. Eight patients discontinued efavirenz due to adverse events prior to measuring plasma efavirenz level and three patients were excluded due to poor adherence. The median (IQR) CD4 cell count was 42 (17-105) cells/mm3 and median (IQR) plasma HIV-1 RNA was 5.8 (5.4-6.3) log copies/mL. Of 139 patients, 38 received anti-tuberculous regimens without rifampicin. At week 12, median (IQR) plasma efavirenz level of all 139 patients was 2.3 (1.4-3.9) mg/dL.

The first three most frequent CYP2B6 SNPs detected were 3003C>T, 785A>G, and 21563C>T, respectively. The nucleotide substitution at positions 499 and 1375 were not detected. Three CYP2B6 SNPs were found to be associated with high plasma efavirenz levels, included 516G>T, 785A>G, and 21563C>T; but 18492T>C was associated with

low plasma efavirenz level. The frequent CYP2B6 haplotype was *1/*6 (41%), *1/*1 (35%), *1/*2 (7%), *6/*6 (7%), *4/*6 (4%), *1/*4 (3%), *2/*4 (1%), *2/*6 (1%), and *5/*6 (1%), respectively. Three of 9 haplotypes identified, including *6/*6, *1/*6, and *5/*6 were associated with high plasma efavirenz levels. There was no CYP2B6 genetic mutation in 35% of all patients that the haplotypes was determined to be *1/*1.

By multivariate analysis, factors associated with low plasma efavirenz level included specific haplotype and high body weight ($P<0.05$) but the factor “receiving rifampicin” is marginal significant. Figure 3 displays the relationship between body weight at week 12 and plasma efavirenz level. By correlation analysis, there appears to be a relationship between high body weight and low plasma efavirenz level ($P<0.05$). Figure 4 shows the scatter plot of plasma efavirenz levels at 12 hours after dosing between the patients who received efavirenz with/without rifampicin and overall cohort. Medians (IQR) plasma efavirenz level of all patients, 101 patients who were concurrently receiving efavirenz and rifampicin, and 38 patients who did not received rifampicin were 2.3 (1.4-3.9) mg/dL, 2.1 (1.3-3.5) mg/dL, and 2.7 (1.8-5.4) mg/dL, respectively. The interpatient variability of plasma efavirenz levels in the corresponding groups were 95%, 75%, and 107%, respectively. Seven of 101 (7%) of the patients who were concurrently receiving rifampicin and 3 of 38 (8%) of those who were not concurrently receiving rifampicin had plasma efavirenz level below the recommended level.

DISCUSSION AND CONCLUSION

Genetic variances among individuals influence metabolism, distribution and elimination of drugs. Higher plasma efavirenz concentrations may result from genetic differences in metabolism of this drug. Efavirenz is metabolized by cytochrome P450 2B6 (CYP2B6), CYP2A6 and UDP-glucuronosyltransferase 2B7 (UGT2B7). However, CYP2B6 is the metabolizing enzyme involved in the metabolism of efavirenz and a genetically polymorphic enzyme is associated with increased plasma efavirenz concentration and a higher incidence of neurotoxicity during initial treatment. The allelic variant 516G > T is associated with diminished activity of the CYP2B6 isoenzyme, increased plasma efavirenz concentrations together with increased incidence of efavirenz associated neuropsychological toxicity.

Several studies have observed associations between CYP2B6 polymorphisms and efavirenz pharmacokinetics and/or treatment response. The studies have found an association between CYP2B6 G516T and higher plasma efavirenz concentrations. Gounden V et al. founded the correlations between CYP2B6 516TT genotype and efavirenz concentrations with increased incidence of fatigue, mood and sleep disorders post initiation of efavirenz. Moreover, previous study establishes that CYP2B6 T983C increases the predictive capability of CYP2B6 G516T for efavirenz pharmacokinetics. Associations between increased plasma efavirenz exposure, CYP2B6 516G>T, and 983T>C have been consistent across multiple studies and populations.

In addition, our studies of CYP2B6 polymorphisms showed significant allelic variants (CYP2B6 c.516G>T and c.785A>G polymorphisms) which may decrease the clearance of efavirenz by reducing the activity of CYP2B6 enzyme and thereby increase efavirenz plasma concentration. Prospective CYP2B6 c.516G>T, c.785A>G and

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c.T983C genotyping has been proposed for identifying patients at risk of neurotoxicity for efavirenz-based antiretroviral therapy in HIV infected patients.

Efavirenz has a low genetic barrier to viral drug resistance, such that a single mutation, most frequently K103N in the reverse transcriptase gene. The development of efavirenz-resistant mutant may be due to repetitive exposure to subtherapeutic drug levels. Treatment failure has been found to be more frequent in patients with low efavirenz trough level, compared with those with high level (>1100 ng/mL). As for efavirenz, some investigators have suggested that the lower limit for the therapeutic range of efavirenz should be raised from 1,000-2,300 ng/mL. The patients where are presented with g.C18492T variants may be exposed to close to subtherapeutic drug concentration. The median efavirenz concentration for patients with the g.18492 heterozygous variants or homozygous variants was significantly lower than those with the wild-type genotype. Repeated exposure to sub-therapeutic concentrations of efavirenz also increases the chance for the development of resistant viral strains and thus treatment failure. The information given by this single-SNP analysis may help to effectively identify HIV-infected individuals who may have a risk for treatment failure. Because the T allele in CYP2B6 g.18492T>C had high frequencies among HIV-infected population, their role as an indicator of clinical outcomes needs to be defined in this population and may have a global impact on HIV/AIDS treatment with the increasing used efavirenz in developing countries.

Integrating CYP2B6 pharmacogenetics in clinical practices

Efavirenz dose reduction or initiation of efavirenz treatment at reduced dose is possible with therapeutic anti-HIV-1 potency promised in CYP2B6 *6/*6 (516TT and 785GG) homozygotes, which could relieve the patients of the efavirenz-associated CNS symptoms. It may also decrease the risk of development of efavirenz-resistant HIV-1, an important issue in developing countries. It is recommended to establish CYP2B6 genotype in patients receiving efavirenz to predict their metabolizing behavior. Accordingly, to obtain efavirenz steady-state concentrations within the therapeutic range (1,000–4,000 ng/mL), it would be advisable to implement a gradual reduction in dose to 400 or 200 mg/day for patients that are intermediate or poor metabolizers, respectively.

Hasse et al. also reported a patient with genotype CYP2B6 516T/T, who had chronic CNS symptoms and extremely high efavirenz concentration at 600 mg dose, but which the symptoms were resolved by reducing the efavirenz dose to 200 mg [15]. Gatananga et al. showed that those patients with the CYP2B6 516G > T SNP had significantly higher plasma efavirenz concentrations (> 6000 ng/mL) on the standard dosing regimen. In that study, the reduction of the initial efavirenz dosages to either 400 mg or 200 mg resulted in the lowering of efavirenz concentrations towards the therapeutic range and an improvement in CNS related symptoms in the majority of these patients. The dosage was thus reduced from 600 to 400 and 200 mg in individuals, and their HIV-1 load was successfully suppressed below detection limit (50 copies/ml) at these dosages. Interestingly, individuals suffered from chronic CNS-related symptoms at the standard dosage, but had an improvement with efavirenz dose reduction. Taken together, the above report suggests that the quality of life of CYP2B6 516T/T genotype

Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

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2. **การนำผลงานวิจัยไปใช้ประโยชน์**

- **เชิงพาณิชย์**

ผลงานวิจัยนี้ มีการนำไปประยุกต์ใช้จริงทางคลินิกแล้ว โดยห้องปฏิบัติการเภสัชพันธุศาสตร์ ศูนย์การแพทยสมเด็จพระเทพรัตน์ โรงพยาบาลรามาธิบดี ได้นำข้อมูลจากการศึกษาวิจัยไปใช้เพื่อการพัฒนาการตรวจวินิจฉัยทางเภสัชพันธุศาสตร์ของยาอิฟาวิเรนซ์ในผู้ติดเชื้อเอชไอวี ทั้งในด้าน

(1) การพัฒนาชุดตรวจวินิจฉัยทางเภสัชพันธุศาสตร์

(2) การพัฒนาวิธีการวัดระดับยาอิฟาวิเรนซ์ในกระแสเลือดผู้ติดเชื้อเอชไอวี

(3) การแปลผล วิเคราะห์ และรายงานผลการตรวจวินิจฉัยทางเภสัชพันธุศาสตร์เพื่อบ่งชี้ขนาดยาที่เหมาะสมในผู้ติดเชื้อเอชไอวี

- **เชิงนโยบาย (มีการกำหนดนโยบายอิงงานวิจัย/เกิดมาตรการใหม่/เปลี่ยนแปลงระเบียบข้อบังคับหรือวิธีทำงาน)**

โดยแพทย์ผู้เชี่ยวชาญด้านโรคติดเชื้อของโรงพยาบาลรามาธิบดี จะทำการสั่งตรวจความหลากหลายทางพันธุกรรมบนยีน CYP2B6 ก่อนเริ่มการรักษาด้วยยาอิฟาวิเรนซ์ในผู้ติดเชื้อเอชไอวีบางรายที่พิจารณาว่าจะมีความเสี่ยง และมีการประเมินผลโดยการสั่งตรวจระดับยาอิฟาวิเรนซ์ในกระแสเลือดผู้ติดเชื้อเอชไอวี ทำให้ดูแลรักษาผู้ติดเชื้อเอชไอวีได้อย่างมีประสิทธิภาพ ลดโอกาสเกิดอาการไม่พึงประสงค์จากการใช้ยา หรือป้องกันการเกิดระดับยาต่ำกว่าการรักษาได้ในผู้ป่วยบางราย

- **เชิงสาธารณะ (มีเครือข่ายความร่วมมือ/สร้างกระแสความสนใจในวงกว้าง)**

การนำเสนอผลงานวิจัยนี้ทางสื่อโทรทัศน์

1. ข่าวช่อง 9 อสมท. ข่าวภาคค่ำ ออกอากาศวันที่ 8 เมษายน 2556
2. ข่าวช่อง 5 รายการ ข่าว 5 หน้า 1 ออกอากาศวันที่
3. ช่อง Voice TV เรื่อง เภสัชกรไทยพบยีนเสี่ยงดื้อยาต้านเอดส์ ออกอากาศวันที่ 6 มีนาคม 2556 เวลา 18:16 น. (http://news.voicetv.co.th/technology/64767.html)
4. ช่องรามาชาแนล (Rama channel) เรื่องยีนส์แพ้ยา ออกอากาศวันที่ 5 กรกฎาคม 2555 http://www.ramachannel.tv/detail.php?id=1507

การนำเสนอผลงานวิจัยทางสื่อสิ่งพิมพ์ต่าง ๆ

1. HIV-drug-resistant gene found in Thailand

Bangkok Post วันที่ 4 มีนาคม 2556
(http://www.bangkokpost.com/news/local/338762/hiv-drug-resisting-gene-discovered-in-thailand)

2. นักวิจัยไทยพบยีนดื้อยาเอดส์รีบจดสิทธิบัตร
เดลินิวส์ วันพุธ ที่ 6 มีนาคม 2556 หน้า 1 (ซ้าย) ต่อ หน้า 18
3. วิจัยพบยีนเสี่ยงดื้อยาต้านเอดส์
เดลินิวส์ วันอังคาร ที่ 5 มีนาคม 2556 หน้า 14 (กลาง)
4. พบยีนเสี่ยงดื้อยาต้านเอดส์
ข่าวสด วันที่ 5 มีนาคม 2556 หน้า 15 (บนขวา)
5. พบยีนเสี่ยงดื้อยาต้านเอดส์
ข่าวสด วันที่ 6 มีนาคม 2556 หน้า 15 (ล่างซ้าย)
6. รามาฯเจ๋งพบยีนเสี่ยงดื้อยาเอดส์ ยื่นจดสิทธิบัตรครั้งแรกของโลก
มติชน วันที่ 5 มีนาคม 2556 หน้า 10 (ซ้าย)
7. รามาฯเจ๋งพบยีนเสี่ยงดื้อยาเอดส์ ยื่นจดสิทธิบัตรครั้งแรกของโลก
มติชน วันที่ 6 มีนาคม 2556 หน้า 10 (ซ้าย)
8. นักวิจัยไทยพบยีนดื้อยาต้านเอดส์
แนวหน้า วันที่ 5 มีนาคม 2556 หน้า 2 (ขวา)
9. รพ.รามาเจ๋ง พบยีนดื้อยาต้านเชื้อเอดส์
ASTV ผู้จัดการรายวัน วันที่ 5 มีนาคม 2556 หน้า 1 (บนขวา) ต่อหน้า 4
10. ครั้งแรกของโลก นักวิจัยไทยเจ๋ง พบยีนเสี่ยงดื้อยาต้านเอดส์
ไทยรัฐ วันที่ 8 กรกฎาคม 2556
11. รู้จักเภสัชพันธุศาสตร์ วิจัยแพ้ยาผ่านยีนระดับโลก
โพสต์ทุเดย์ 27 เมษายน 2556
12. รามาเจ๋งวิจัยพบยีนเสี่ยงดื้อยาต้านเอดส์
มหิดลสาร ปีที่ 38 ฉบับที่ 3 (31 มีนาคม 2556)

- **เชิงวิชาการ (มีการพัฒนาการเรียนการสอน/สร้างนักวิจัยใหม่)**

3. อื่นๆ (เช่น ผลงานตีพิมพ์ในวารสารวิชาการในประเทศ การเสนอผลงานในที่ประชุมวิชาการ หนังสือ การจดสิทธิบัตร)

3.1 งานเขียนหนังสือ 1 บท

โดยนำองค์ความรู้ที่ได้จากการศึกษาวิจัยนี้ไปใช้ในการเรียบเรียงบทความเรื่อง เภสัชพันธุศาสตร์และการแพทย์เฉพาะบุคคล (PHARMACOGENOMICS AND

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PERSONALIZED MEDICINE) ซึ่งเป็นบทหนึ่งในหนังสือ พยาธิวิทยาคลินิก ฉบับพิมพ์ครั้งที่ 2 จำนวน 1,000 เล่มโดยหนังสือเล่มนี้มีการเผยแพร่ และใช้สำหรับการเรียนการสอนนักศึกษาแพทยศาสตร์ เทคนิคการแพทย์ และผู้สนใจหลากหลายสาขา

3.2 Poster presentation 3 ครั้ง

a. Optimizing Efavirenz Treatment by Detection of Pharmacogenetic Markers of CYP2B6 Corrected with Plasma Concentration in HIV-1 Infections. The 12th International Congress of Human Genetics and the 61st Annual Meeting of the American Society of Human Genetics, American Society of Human Genetics (ASHG) วันที่ 11-15 ตุลาคม 2554 ณ Le Palais des Congrès, Montreal, Quebec ประเทศแคนาดา

b. A pharmacogenetic and pharmacokinetic study of efavirenz in HIV-1 infection: subtherapeutic concentrations of EFV from CYP2B6 polymorphism. Pharmacogenomics and Personalised Medicine 2011 วันที่ 27 กันยายน -4 ตุลาคม 2554 ณ Wellcome Trust Genome Campus, Hinxton, Cambridge ประเทศสหราชอาณาจักรบริเตนใหญ่ (UK)

c. Low efavirenz pharmacokinetics in HIV-1 infected Thai adults are associated with CYP2B6 polymorphism. Keystone Symposia on Molecular and Cellular Biology, The Human Genomics and Personalized Medicine
วันที่ 17–21 กรกฎาคม 2556 Stockholm ประเทศสวีเดน

3.3 Proceeding พร้อมนำเสนอปากเปล่า

CYP2B6 GENOTYPING FOR OPTIMIZING EFAVIRENZ TRETAMENT IN HIV-1 INFECTION. Pattamawan Prapaithong, Ekawat Pasomsub, Chutatip Srichunrusami, Tim R. Cressey, Wasun Chantratita, Chonlaphat Sukasem* รวมผลงานประชุมวิชาการพันธุศาสตร์แห่งชาติ ครั้งที่ 17 (Proceeding of the 17th National Genetic Conference) การวิจัยพันธุศาสตร์เพื่อการแปลผลสู่การประยุกต์ (Translational genetic research for application) สมาคมพันธุศาสตร์แห่งประเทศไทย วันที่ 7-9 เมษายน 2554 ณ โรงแรมอิมพี่เรียลแม่ปิง จ.เชียงใหม่ ประเทศไทย ISBN 978-974-672-599-6

3.4 การบรรยายในการประชุมวิชาการระดับนานาชาติและระดับชาติ งานประชุมแพทย์ และการฝึกอบรมเชิงปฏิบัติการ

a. การประชุมวิชาการระดับนานาชาติ

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1st Meeting South East Asian Pharmacogenomics Research Network (SEAPharm) Symposium for Genetic and Genome-Guided Personalized Medicine in Asia: Overview and Applications ณ โรงแรมสยามซิตี้ กรุงเทพ ในวันที่ 2-3 กุมภาพันธ์ 2555

หัวข้อบรรยาย: Clinical application of pharmacogenetics testing in HIV infection

b. การประชุมวิชาการระดับชาติ และการประชุมแพทย์ของโรงพยาบาลต่างๆ

c. การฝึกอบรมเชิงปฏิบัติการเรื่อง Implementation of Innovative Pharmacogenetic Testing into Practice ณ ห้องปฏิบัติการเภสัชพันธุศาสตร์และการแพทย์เฉพาะบุคคล ศูนย์การแพทย์สมเด็จพระเทพรัตน์โรงพยาบาลรามาธิบดี วันที่ 30-31 พฤษภาคม 2555

3.5 เอกสารประกอบการสอน

a. รายวิชา SCID 333 สำหรับนักศึกษาหลักสูตรแพทยศาสตรบัณฑิต คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี และวิทยาลัยแพทยศาสตร์ วชิรพยาบาล

b. รายวิชา RAPA 611 สำหรับนักศึกษาปริญญาโท และปริญญาเอก หลักสูตรพยาธิวิทยาคลินิก

c. สำหรับการฝึกอบรมแพทย์ประจำบ้าน สาขาพยาธิวิทยาคลินิก สาขาอายุรศาสตร์ (โรคติดเชื้อ) และสาขากุมารเวชศาสตร์

3.6 การจดสิทธิบัตร

อยู่ในขั้นศึกษาข้อมูล และพิจารณาประเด็นที่จะทำการการจดสิทธิบัตร เช่น การพัฒนาการตรวจ การแปล วิเคราะห์ และรายงานผลการตรวจวินิจฉัยทางเภสัชพันธุศาสตร์เพื่อบ่งชี้ขนาดยาที่เหมาะสมในผู้ติดเชื้อเอชไอวี

ภาคผนวก (สรุป)

ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

ภาคผนวก 1: Sukasem C, Prapaithong P., Pasomsub E., Srichunrusami C., Cressey TR., Jantararoungtong T., Chantratita W. Pharmacogenetic markers of CYP2B6 associated with efavirenz plasma concentrations in HIV-1 infected Thai adults.Br J Clin Pharmacol. 2012 Dec;74(6):1005-12.

ภาคผนวก 2: Sukasem C, Chamnanphol M, Koomdee N, Puangpetch A, Santon S, Jantararoungtong T, Prommas S, Manosuthi W. High Plasma Efavirenz Concentration and CYP2B6 Polymorphisms in Thai HIV-1 Infections. Drug Metab Pharmacokinet. 2013 Oct; 28 (5).

ภาคผนวก 3: Sukasem C and Sungkanuparph S. Would a CYP2B6 test help HIV patients being treated with Efavirenz?, Pharmacogenomics. 2013. Pharmacogenomics. 2013 Jul;14(9):999-1001.

ภาคผนวก 4: Sukasem C, Manosuthi W, Koomdee N, Santon S, Jantararoungtong T, Prommas S, Chamnanphol M, Puangpetch A. Low efavirenz pharmacokinetics in HIV-1 infected Thai adults are associated with CYP2B6 polymorphism. 2013. (submitted)

ภาคผนวก 5: Manosuthi W, Sukasem C, Lueangniyomkul A, Mankatitham W, Thongyen S, Nilkamhang S, Manosuthi S, Sungkanuparph S. Impact of Pharmacogenetic Markers of CYP2B6, Clinical Factors, and Drug-Drug Interaction on Efavirenz Levels in HIV/Tuberculosis Co-Infected Patients. Antimicrob Agents Chemother. 2013 Feb;57(2):1019-24.

ภาคผนวก 6: Manosuthi W, Sukasem C, Mankatitham W, Lueangniyomkul A, Thongyen S, Nilkamhang S, Manosuthi S, Sungkanuparph S. CYP2B6 18492T>C Polymorphism Compromises Efavirenz-based Antiretroviral Regimen in Co-infected HIV and Tuberculosis Patients Carrying CYP2B6 Haplotype *1/*1. 2013. (submitted)

ภาคผนวก 7: Manosuthi W, Sukasem C, Mankatitham W, Lueangniyomkul A, Thongyen S, Nilkamhang S, Manosuthi S, Sungkanuparph S. CYP2B6 Haplotype and Biological Factors Responsible for Hepatotoxicity in HIV-infected Patients Receiving Efavirenz-based Antiretroviral Therapy. 2013. (submitted)

การนำเสนอผลงานวิจัยนี้ทางสื่อโทรทัศน์

ภาคผนวก 8: ข่าวช่อง 9 อสมท. ข่าวภาคค่ำ ออกอากาศวันที่ 8 เมษายน 2556

ภาคผนวก 9: ข่าวช่อง 5 รายการ ข่าว 5 หน้า 1 ออกอากาศวันที่

ภาคผนวก 10: Voice TV เรื่อง เภสัชกรไทยพบยีนเสี่ยงดื้อยาต้านเอดส์ ออกอากาศวันที่ 6 มีนาคม 2556 เวลา 18:16 น. (http://news.voicetv.co.th/technology/64767.html)

ภาคผนวก 11: ข่าวช่องรามาชาแนล (Rama channel) เรื่องยีนส์แพ้ยา ออกอากาศวันที่ 5 กรกฎาคม 2555 http://www.ramachannel.tv/detail.php?id=1507

การนำเสนอผลงานวิจัยทางสื่อสิ่งพิมพ์ต่าง ๆ

ภาคผนวก 12: นักวิจัยไทยพบยีนดื้อยาเอดส์รีบจดสิทธิบัตร-เดลินิวส์ วันพุธ ที่ 6 มีนาคม 2556 หน้า 1 (ซ้าย) ต่อ หน้า 18

ภาคผนวก 13: วิจัยพบยีนเสี่ยงดื้อยาต้านเอดส์-เดลินิวส์ วันอังคาร ที่ 5 มีนาคม 2556 หน้า 14 (กลาง)

ภาคผนวก 14: พบยีนเสี่ยงดื้อยาต้านเอดส์-ข่าวสด วันที่ 5 มีนาคม 2556 หน้า 15 (บนขวา)

ภาคผนวก 15: พบยีนเสี่ยงดื้อยาต้านเอดส์-ข่าวสด วันที่ 6 มีนาคม 2556 หน้า 15 (ล่างซ้าย)

ภาคผนวก 16: รามาฯเจ๋งพบยีนเสี่ยงดื้อยาเอดส์ ยื่นจดสิทธิบัตรครั้งแรกของโลก-มติชน วันที่ 5 มีนาคม 2556 หน้า 10 (ซ้าย)

ภาคผนวก 17: รามาฯเจ๋งพบยีนเสี่ยงดื้อยาเอดส์-ยื่นจดสิทธิบัตรครั้งแรกของโลก มติชน วันที่ 6 มีนาคม 2556 หน้า 10 (ซ้าย)

ภาคผนวก 18: นักวิจัยไทยพบยีนดื้อยาต้านเอดส์-แนวหน้า วันที่ 5 มีนาคม 2556 หน้า 2 (ขวา)

ภาคผนวก 19: รพ.รามาเจ๋ง พบยีนดื้อยาต้านเชื้อเอดส์-ASTV ผู้จัดการรายวัน วันที่ 5 มีนาคม 2556 หน้า 1 (บนขวา) ต่อหน้า 4

ภาคผนวก 20: ครั้งแรกของโลก นักวิจัยไทยเจ๋ง พบยีนเสี่ยงดื้อยาต้านเอดส์-ไทยรัฐ วันที่ 8 กรกฎาคม 2556

ภาคผนวก 21: รู้จักเภสัชพันธุศาสตร์ วิจัยแพ้ยาผ่านยีนระดับโลก-โพสต์ทูเดย์ 27 เมษายน 2556

ภาคผนวก 22: รามาเจ๋งวิจัยพบยีนเสี่ยงดื้อยาต้านเอดส์-มหิดลสาร ปีที่ 38 ฉบับที่ 3 (31 มีนาคม 2556)

ภาคผนวก 23: รู้ข่าวก้าวทันโลก-รามาฯเจ๋งพบยีนเสี่ยงดื้อยาต้านเอดส์ครั้งแรกของโลก (http://healthnewsdaily.blogspot.com/2013/03/blog-post.html

BCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2012.04278.x

Pharmacogenetic markers of CYP2B6 associated with efavirenz plasma concentrations in HIV-1 infected Thai adults

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Keywords
CYP2B6, efavirenz, HIV-1, pharmacogenetics, SNPs, Thailand

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Interindividual variability in efavirenz plasma concentrations is associated with *CYP2B6* genetic polymorphisms.
- Twenty-nine different alleles of the *CYP2B6* gene are listed. *CYP2B6 *6*, *9*, *16*, *26*, *27* and *28* carriers are reported to be associated with slower efavirenz oral clearance.
- The allelic variant 516G>T is associated with diminished activity of CYP2B6 and high efavirenz plasma concentrations are associated with an increase risk of neuropsychological toxicity.

WHAT THIS STUDY ADDS

- This study identified three SNPs in the *CYP2B6* gene which could potentially act as additional independent predictors of efavirenz plasma concentrations beyond that provided by the *CYP2B6* c.516G>T polymorphism.
- The *GAC-CYP2B6* haplotype (G516T/A785G/C21563T) is associated with higher plasma efavirenz concentrations in HIV-infected Thai adults.
- The *CYP2B6* g.18492 T>C polymorphism was significantly associated with lower efavirenz concentrations than those with the homozygous wild-type.

Aims

To investigate the frequency of *CYP2B6* polymorphisms and the influence of haplotype structure on plasma efavirenz concentrations in Thai adults with HIV-1 infection.

Methods

Genotyping of nine single nucleotide polymorphisms (SNPs, c.64C>T, c.499C>G, c.516G>T, c.785A>G, c.1375A>G, c.1459C>T, g.3003T>C, g.18492C>T and g.21563C>T) of *CYP2B6* were performed using real-time PCR-based allelic discrimination on blood samples from 52 HIV-infected adults who had received an efavirenz-based regimen. Plasma efavirenz concentrations were measured by high performance liquid chromatography.

Results

The minor allele frequencies for c.64C>T, c.516G>T, c.785A>G, g.3003C>T, g.18492T>C and g.21563C>T were 0.087, 0.365, 0.413, 0.308 and 0.356, respectively. However, no variant alleles were identified for three SNPs (c.499 C>G, c.1375 A>G and c.1459 C>T). Efavirenz plasma concentrations were significantly associated with c.516G>T ($P = 0.0095$), c.785A>G ($P = 0.0017$), g.21563C>T ($P = 0.0036$) and g.18492C>T ($P = 0.0011$). The composite *CYP2B6* of three SNPs (c.516G $\geq$ T, c.785A $\geq$ G and g.21563C $\geq$ T) genotypes were significantly associated with higher efavirenz concentrations.

Conclusions

Our data indicate that the GAC-*CYP2B6* haplotype is associated with higher plasma efavirenz concentrations in HIV-infected Thai adults.

Introduction

Efavirenz is potent and effective non-nucleoside reverse transcriptase inhibitor (NNRTI) that is commonly used as part of the first line antiretroviral regimen for the treatment of HIV/AIDS. Efavirenz-containing highly active antiretroviral therapy (HAART) is also preferred in patients with tuberculosis co-infection requiring rifampicin-containing therapy [1]. Although its safety profile is considered satisfactory, central nervous system (CNS) side effects are commonly reported. The CNS side effects range from headaches and dizziness to insomnia, hallucinations, acute mania and psychosis [2]. The exact mechanism responsible for CNS toxicity associated with efavirenz remains unknown [2, 3], but high plasma concentrations have been reported to be a predictor of early neuropsychological disturbances in patients initiating an efavirenz containing antiretroviral regimen [2, 4–6]. Significant inter-individual variability in efavirenz plasma concentrations in adults and children has been observed [2, 3, 7, 8]. Mid-dose or trough efavirenz plasma concentrations below 1000 ng ml^{-1} have been associated with an increased risk of virological failure [2, 8, 9], whereas concentrations above 4000 ng ml^{-1} have been associated with a higher risk of CNS side effects [2, 8].

Efavirenz is metabolized primarily by the hepatic CYP2B6 enzyme to form 8-hydroxy and 7-hydroxy efavirenz metabolites, with minor contributions from CYP3A4/5 and CYP2A6 [10, 11]. The *CYP2B6* gene is highly polymorphic and has been mapped to chromosome 19, which is 28 kb long and consists of nine exons [12]. Previous studies have shown that inter-individual variability in efavirenz plasma concentrations is associated with the *CYP2B6* genetic polymorphisms [5, 8, 13, 14]. Currently, 29 different alleles of the *CYP2B6* gene are listed. *CYP2B6 *6*, **9*, **16*, **26*, **27* and **28* carriers have been reported to be associated with slower efavirenz oral clearance [14–18]. The most commonly studied allele is **6* (c.516G ≥ T and c.785A ≥ G). The **6* allele is associated with higher efavirenz plasma concentrations and an increased risk of CNS toxicity [15, 16]. The allelic variant c.516G>T is associated with diminished activity of CYP2B6, increased efavirenz plasma concentrations and neuropsychological toxicity [2, 5, 7, 15].

Minimizing drug associated toxicity is a major challenge. It is thought that pharmacogenetic information may be able to help identify patients at an increased risk of drug toxicity. Recently, it has been reported that *CYP2B6*-516G>T significantly affected the drug metabolism of efavirenz in HIV-infected Thai children and high concentrations were associated with psychiatric side effects [7]. The objective of this study was to determine the frequency of nine *CYP2B6* polymorphisms in HIV-infected Thai adults and identify SNPs and/or haplotypes in this population that are associated with efavirenz plasma concentrations.

Methods

Sample collection

This retrospective analysis used stored plasma and cell pellets that were collected from HIV infected Thai adults followed in a prospective observational cohort study (http://www.clinicaltrials.gov, NCT00433030). HIV-infected patients receiving two NRTIs plus efavirenz for at least 4 weeks with plasma and cell pellet samples available were included. The first plasma sample available after 4 weeks of treatment was selected for analysis. Patients receiving concomitant treatments that could potentially affect efavirenz pharmacokinetics were excluded. All subjects provided written informed consent within the cohort study, which included the use of stored samples for future research following specific ethical clearance. This specific retrospective analysis, performed on anonymized DNA and plasma samples, was approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi Hospital (ID 06–51-31) and the Faculty of Associated Medical Sciences, Chiang Mai University (Sor Thor 6393(4)/Vor Jor 202).

Sample preparation and DNA extraction

Peripheral blood samples were obtained and the plasma was aliquoted and frozen within 1 h of collection at –20°C. For each blood draw the remaining EDTA cell pellets were stored at –20°C. DNA was isolated from the stored EDTA cell pellets using the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genomic DNA was quantified by a u.v. spectrophotometer ND-1000 at 260 nm (NanoDrop Technologies, Wilmington, DE).

CYP2B6 genotyping

A total of nine SNPs within *CYP2B6* were genotyped (GeneBank accession number NG_000008.7). SNPs c.516G ≥ T (rs3745274) and c.785A ≥ G (rs2279343) have previously been reported to influence enzyme activity and efavirenz concentrations [14] and three *CYP2B6* tagSNPs, g.3003T ≥ C (rs8100458), g.18492C ≥ T (rs2279345) and g.21563C ≥ T (rs8192719), identified using HapMap (http://www.hapmap.org) data on Japanese and Han Chinese populations with an $r^2 \geq 0.8$ were also included [19]. The c.499C ≥ G (rs3826711) SNP, part of the *26 allele, was associated with high efavirenz plasma concentrations in Japanese subjects, and three other SNPs also assessed in this study, c.64C ≥ T (rs8192709), c.1375A ≥ G (rs3211369) and c.1459C ≥ T (rs3211371) were also selected for assessment [15].

Pre-designed TaqMan assays (Applied Biosystems, Foster City, CA) were used to genotype the *CYP2B6* g.3003T>C (assay ID C__2818167_10), c.516G>T (assay ID C__7817765_60), g.18492C>T (assay ID C__26823975_10), g.21563C>T (assay ID C__22275631_10), c.64C>T (assay ID C__2818162_20), c.499C>G (assay ID C__2752377_10), c.1375A>G (assay ID C__2741754_10) and c.1459C>T

(assay ID C__30634242_40). The *CYP2B6* c.785A>G were performed by custom TaqMan assays (Applied Biosystems). The sequences of primers and probes were: *CYP2B6* c.785A>G, TGGAGAAGCACCGTGAAACC (forward), TGGAGCAGGTAGGTGTCGAT (reverse), VIC-CCCCCAAGGACCTC-MGB (wild-type), FAM-CCCCAGGGACCTC-MGB (mutant).

For SNP analysis, these nine SNPs were genotyped using an allele-specific fluorogenic 5′ nuclease chain reaction assay with predesigned primers and TaqMan MGB probes (TaqMan SNP Genotyping Assay; Applied Biosystems, Foster City, CA). Sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest used two TaqMan MGB probes. One probe was labelled with VIC dye and detected the Allele 1 sequence and the second probe was labelled with FAM dye and detected the Allele 2 sequence.

Measurement of efavirenz plasma concentrations

Blood samples were centrifuged and the plasma was aliquoted and frozen within 4 h of collection at −20°C. Efavirenz plasma drug concentrations were measured at the Faculty of Associated Medical Sciences, Chiang Mai University using an isocratic reversed-phase high performance liquid chromatography (HPLC) method with ultraviolet detection at 245 nm [7]. Briefly, patient plasma samples (300 µl) and all calibration and control samples were heat inactivated in a water bath at 56°C for 30 min prior to assay. Sample pretreatment involved protein precipitation with acetonitrile (360 µl) and following centrifugation the sample supernatant was injected into the HPLC machine. Chromatography was performed using an Agilent 1100 HPLC machine with an Omnispher C18 (150 × 4.6 mm i.d./particle size 5 µm) analytical column (Varian, CA, USA), a Chromquard RP guard column and a mobile phase consisting of 10 mM KH_2PO4 pH 3.1 : acetonitrile (50 : 50, v/v). This method was validated using the AIDS Clinical Trials Group (ACTG) method validation guidelines [6] over the concentration range of 78–10 000 ng ml^{-1}. The average accuracy was 102–105% and precision (both inter- and intra-assay) was ≤5% of the coefficient of variation (CV). Overall extraction recovery was 106% and efavirenz was stable under various storage conditions. Plasma samples with efavirenz concentrations ≥10 000 ng ml^{-1} were diluted and re-assayed. This laboratory participates in the international external quality Pharmacology Quality Control (Precision Testing) programme of the AIDS Clinical Trial Group, USA.

Statistical analysis

All genotype distributions were tested for Hardy–Weinberg equilibrium using exact tests. SNPs with a call rate < 95% were omitted. Tagging of SNPs was analyzed using Haploview 4.2 software. A Kruskal–Wallis test was used to test for significant difference in efavirenz concentrations between genotypes. Mann–Whitney U-tests were used to compare efavirenz concentrations between two genotypes. The QTLHAPLO program was used to identify the association between the *CYP2B6* haplotypes and the efavirenz plasma concentration. Statistical significance was defined as $P < 0.05$.

Results

Study population

Samples from 68 subjects were selected for assessment. Fifty-two subjects had detectable efavirenz concentrations and were genotypes for nine *CYP2B6* SNPs. All individuals were >18 years of age and taking 600 mg of efavirenz once daily for at least 4 weeks. Efavirenz plasma concentrations were measured at an average of 13 h after last drug intake. Patient characteristics are summarized in Table 1.

Frequencies of CYP2B6 genetic polymorphisms

The genotype of the nine *CYP2B6* SNPs could be determined for all 52 subjects and the frequencies of each SNP are summarized in Table 2. All *CYP2B6* polymorphisms were found to be in Hardy–Weinberg equilibrium ($P > 0.05$). The minor allele frequencies (MAFs) for c.64C>T, c.516G>T, c.785A>G, g.3003C>T, g.18492T>C and g.21563C>T were 0.087, 0.365, 0.413, 0.308 and 0.356, respectively. No variant alleles were identified for three SNPs (c.499 C>G, c.1375 A>G and c.1459 C>T) in these subjects.

Assessment of individual CYP2B6 polymorphisms with efavirenz plasma concentrations

In the entire cohort, the median (range) efavirenz plasma concentration was 2622 ng ml^{-1} (IQR 1727–3983). Efavirenz plasma concentrations in patients with either the 516GT or TT genotype were significantly higher than in

Table 1

Patient characteristics

Characteristic*	Total (n = 52)
Gender Male : Female (%)	24 : 28
Age (years)	33 (18–53)
Weight (kg)	54 (33–72)
HAART regimen, n (%)	
TDF + FTC + EFV	21 (40)
ZDV+3TC + EFV	17 (33)
D4T+3TC + EFV	6 (11)
ZDV + DDI + EFV	4 (8)
D4T + DDI + EFV	2 (4)
3TC + DDI + EFV	2 (4)
Duration of HAART (weeks)	26 (4–269)
Duration between blood sampling and last intake (h)	13 (10–18)

*Median (range), unless otherwise stated. TDF, tenofovir; FTC, emtricitabine; EFV, efavirenz; ZDV, zidovudine; 3TC, lamivudine; D4T, stavudine; DDI, didanosine.

Table 2

Relationship between *CYP2B6* polymorphisms and efavirenz plasma concentrations

Genetic polymorphism	*n* (%) *n* = 52	Minor allele frequency	Efavirenz plasma concentration (ng ml^{-1}), Median (IQR)
CYP2B6 c.64C>T(rs8192709)			
CC	43 (0.827)		2694 (1704–4192)
CT	9 (0.173)	T = 0.087	2049 (1621–2880)
TT	0 (0.0)		–
P value			0.371
CYP2B6 c.499C>G(rs3826711)			
CC	52 (1.0)		2622 (1721–3983)
CG	0 (0.0)	G = 0.000	–
GG	0 (0.0)		–
P value			–
CYP2B6 c.516G>T(rs3745274)			
GG	22 (0.423)		1902 (1257–3051)
GT	22 (0.423)	T = 0.365	2691 (1818–3500)
TT	8 (0.153)		8422 (4068–10 265)
P value			0.0095
CYP2B6 c.785A>G(rs2279343)			
AA	19 (0.365)		1902 (1304–3157)
AG	23 (0.442)	G = 0.413	2274 (1704–3268)
GG	10 (0.192)		7402 (3011–9965)
P value			0.0017*
CYP2B6 c.1375A>G(rs3211369)			
AA	52 (1.0)		2622 (1721–3983)
AG	0 (0.0)	G = 0.000	–
GG	0 (0.0)		–
P value			–
CYP2B6 c.1459C>T(rs3211371)			
CC	52 (1.0)		2622 (1721–3983)
CT	0 (0.0)	T = 0.000	–
TT	0 (0.0)		–
P value			–
CYP2B6 g.3003C>T(rs8100458)			
CC	25 (0.48)		2806 (1992–5190)
CT	22 (0.423)	T = 0.308	2113 (1482–4160)
TT	5 (0.096)		2366 (889–4547)
P value			0.1454
CYP2B6 g.18492T>C(rs2279345)			
TT	30 (0.577)		2986 (1845–6097)
TC	20 (0.385)	C = 0.231	2012 (1589–2964)
CC	2 (0.038)		1668 (-)†
P value			0.0011
CYP2B6 g.21563C>T(rs8192719)			
CC	22 (0.423)		1902 (1257–3051)
CT	23 (0.442)	T = 0.356	2694 (1856–4149)
TT	7 (0.135)		7908 (3126–10 415)
P value			0.0036

*$P < 0.05$ when comparing 785GG with 785AA and 785AG. †Unable to calculate for IQR, statistical significance was indicated by a Kruskal–Wallis test.

those patients with the homozygous wild-type genotype ($P = 0.0095$, Figure 1A). Subjects with a nucleotide substitution of c.785A>G were observed to have higher efavirenz plasma concentrations than the recommended therapeutic range (1000–4000 ng ml^{-1}) [2] whereas, patients who carried the homozygous variant form (785GG) demonstrated higher efavirenz concentrations than other genotypes (785AA or AG) ($P = 0.0017$, Figure 1B). For SNPs at positions c.64 C ≥ T and g.3003C ≥ T, the efavirenz concentrations were not significantly different ($P = 0.371$ and 0.1454, respectively). The homozygous mutant polymorphisms of g.18492 T ≥ C had a reverse effect to the other SNPs. Efavirenz plasma concentrations for patients with the g.18492 TC (2012 ng ml^{-1}, $n = 20$) or CC (1668 ng ml^{-1}, $n = 2$) genotype were significantly lower than those with the homozygous wild-type (TT; 2986 ng ml^{-1}, $n = 30$) ($P = 0.0011$, Figure 1C). Patients carrying at least one defect allele for the *CYP 2B6* g.21563C>T polymorphism showed higher efavirenz exposure compared with the homozygous wild-type ($P = 0.0095$, Figure 1D).

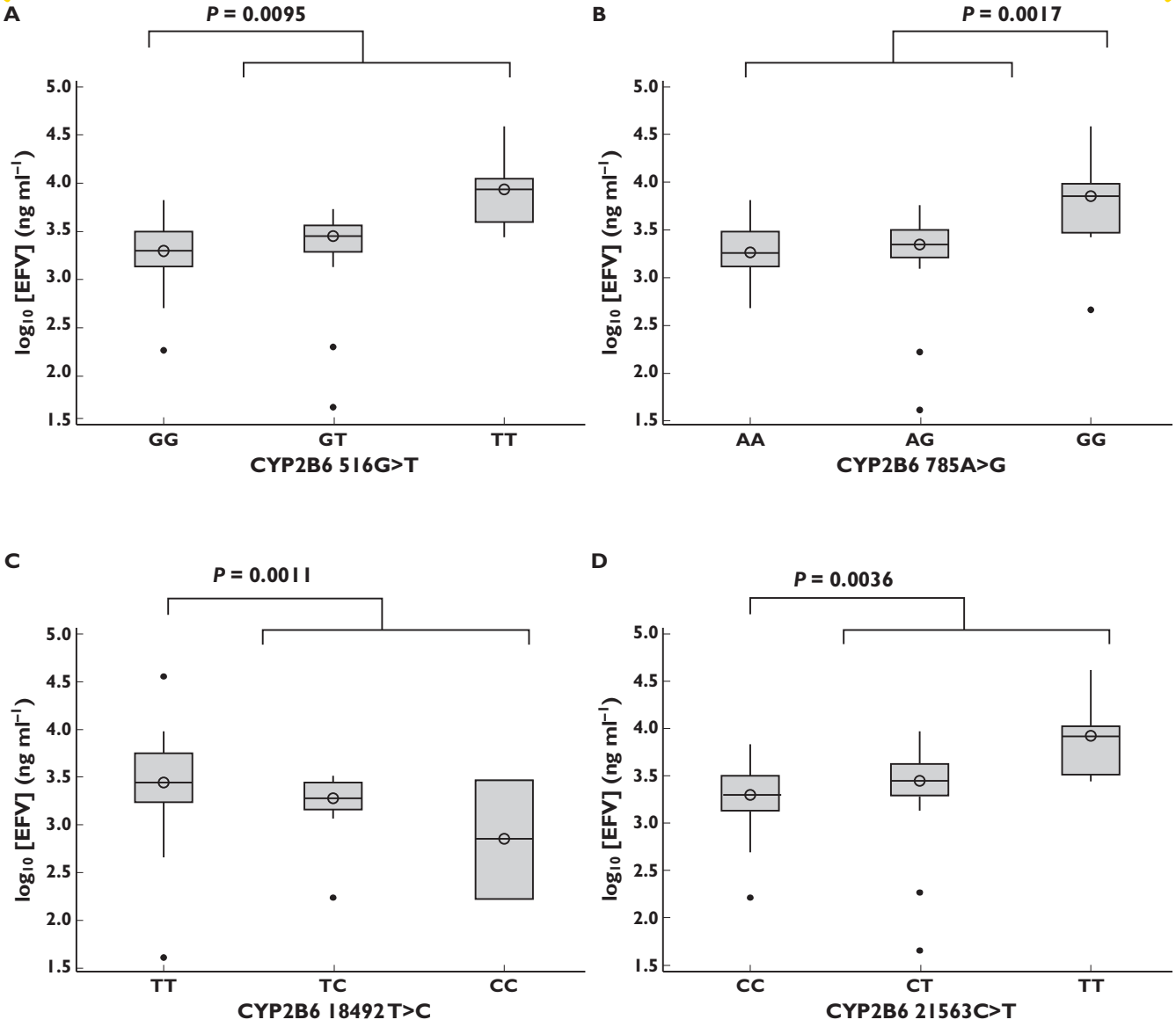

Figure 1

Impact of CYP2B6 polymorphisms; 516G>T (A), 785A>G (B), 18492T>C (C) and 21563C>T (1D) on efavirenz (EFV) plasma concentrations. Middle bar indicates the median and grey bar indicates the interquartile range. Mann–Whitney tests were used to compare efavirenz concentrations between two genotypes

Assessment of CYP2B6 haplotypes and efavirenz plasma concentrations

Significant linkage disequilibrium (LD) in each variant position (*CYP 2B6* c.64C>T, c.516G>T, c.785A>G, g.3003 C>T, g.18492T>C and g.21563C>T) exists. From a pairwise tagging analysis (Haploview, $r^2 > 0.8$), *CYP 2B6* c.516G>T can be representative of c.785A>G and 21563C>T due to strong LD. While, other SNPs (c.64C>T, g.3003 C>T and g.18492T>C) could not capture any SNPs (four SNPs in four tests captured six of six alleles at $r^2 \geq 0.8$; mean maximum r^2 is 0.963, Figure 2).

From single marker association analysis, three SNPs (c.516G>T, c.785A>G and g.21563C>T) were significantly associated with efavirenz plasma concentrations that exceeded the therapeutic range ($P = 0.0012$, 0.0006 and 0.0036, respectively; use Bonferroni correction, $P < 0.0083$). These three SNPs were therefore used to construct a haplotype block. The frequencies of HIV-infected patients with the *CYP2B6*-GAC (516G ≥ T, 785A ≥ G and 21563C ≥ T) were 36.5% ($n = 19$), 40.4% ($n = 21$) and 23.1% ($n = 12$) for non-GAC, GAC heterozygous and GAC homozygous genotypes, respectively. Median (range) efavirenz plasma concentration were 1934 ng ml^{-1} (1313–3520 ng ml^{-1}), 2688 ng ml^{-1} (1780–3276 ng ml^{-1}) and 5544 ng ml^{-1} (2372–9595 ng ml^{-1}) for patients with non-GAC, GAC heterozygous and GAC homozygous genotypes, respectively. This haplotype was significantly associated with higher efavirenz plasma concentrations ($P = 0.0179$, Figure 3).

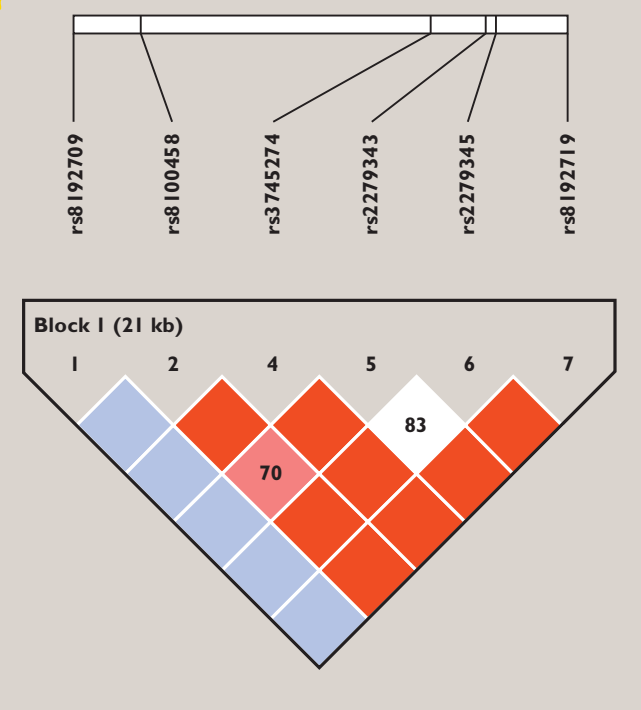

Figure 2

Linkage disequilibrium of *CYP2B6* for each polymorphism, data represents six SNPs in Haploview 4.2 software. Red quadrate without D′ worth display mean D′ = 1, LOD ≥ 2 while pale blue square mean D′ = 1, LOD < 2; white quadrate, D′ < 1 and LOD < 2; pale red square, D′ < 1 and LOD ≥ 2

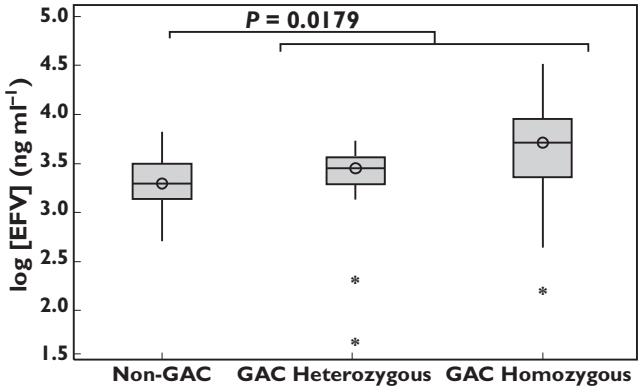

Figure 3

CYP2B6 GAC haplotype. Middle bar indicates the median and grey box indicates the interquartile range. Asterisks represent an individual subject

Discussion

SNPs within the *CYP2B6* gene contribute towards the high inter-individual variability in efavirenz plasma concentrations [5, 8, 13, 14]. This retrospective study was aimed at assessing the frequency of nine *CYP2B6* SNPs in HIV-infected Thai adults to determine which could act as predictors of efavirenz plasma concentrations, in addition to that provided by the well-studied 516G>T polymorphism [7, 20–22].

Overall, the detected frequencies of the nine SNPs were in good agreement with published data [15, 19]. The most common SNP identified in this study was the c.785A>G (MAF = 0.413), while c.516G>T was the second most frequent ((MAF = 0.365). Both these polymorphisms were more common than in Japanese and Chinese HIV-infected patients [15, 20]. The nucleotide substitutions c.499C ≥ G, c.1375A ≥ G and c.1459C ≥ T were absent in this cohort, whereas a previous study showed a low frequency among the Japanese group [15]. The allelic frequency of the *CYP2B6* SNPs in this study was similar to that reported in Thai HIV-infected children and adults [7, 19].

In agreement with previous studies, we observed a significant gene dose effect between c.516G>T and efavirenz [7, 23–25]. In addition, c.785A>G and g21563C>T genotypes were also associated with efavirenz plasma concentrations. Conversely, the variant allele of 18492T>C was associated with lower plasma concentrations of efavirenz. However, the plasma efavirenz concentrations in all variants of those SNPs were within the therapeutic range. The clinical significance of 18492T>C polymorphisms with respect to lower efavirenz concentrations is unknown [22] and further studies with a larger sample are required.

To our knowledge, the association of c.516G ≥ T with efavirenz pharmacokinetics is well established [26–28] but the impact of *CYP2B6* haplotype, GAC (516G, 785A and g.21563C) on steady-state efavirenz plasma concentrations is not well-defined. It is unclear why the g.21563C>T polymorphisms, which are unlikely to impact on the functional effect of *CYP2B6,* were associated. However, it has been proposed that g.21563C>T is linked to 785A>G, which is correlated with reduced CYP2B6 activity [14–16]. Due to the complexity of the *CYP2B6* polymorphisms, it is likely that haplotypes rather than a single polymorphism would be a better predicator of efavirenz plasma concentrations.

There are several limitations to our study. Firstly, the study population is small and the SNPs and haplotypes identified in this study need to be confirmed in a larger population. Also, the retrospective analysis creates several possible biases in the selection of subjects. Specifically; subjects who did not tolerate efavirenz may have been excluded due to switching prior to the samples being obtained. Finally, the sampling time varied from 10 to 18 h after efavirenz administration, which might impact on the interpretation of the efavirenz plasma concentration.

In conclusion, the results of the present study suggest that efavirenz plasma concentrations are significantly higher in HIV-infected Thai adults with *CYP2B6*-c.516G>T, -c.785A>G and -g.21563C>T. Also, multiple SNPs (*CYP2B6* haplotype, GAC) may have potential value in predicting efavirenz concentrations and should be investigated to see

if they can help identify patients who are at an increased risk of adverse drug reactions.

Competing Interests

There are no competing interests to declare.

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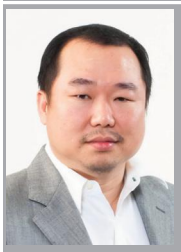
Would a *CYP2B6* test help HIV patients being treated with efavirenz?

"*Therefore, a genotyping test for common functional variants of* CYP2B6*6, *which contains both the 516G>T and 785A>G polymorphisms, prior to the initiation of therapy is recommended for identifying patients at risk of efavirenz-associated neurotoxicity in clinical practice.*"

KEYWORDS: CYP2B6 ■ efavirenz ■ HIV ■ pharmacogenetics ■ polymorphism ■ toxicity

Chonlaphat Sukasem



Somnuek Sungkanuparph

Author for correspondence:

Efavirenz is a potent and effective non-nucleoside reverse transcriptase inhibitor that is a preferred component of first-line antiretroviral therapy (ART) for HIV-1-infected individuals in both wealthy and resource-limited countries [1,2]. The use of efavirenz in clinical practice has further increased in recent years, especially in developing countries. It is usually prescribed at a fixed dosage of 600 mg once daily. Some patients who receive efavirenz have experienced adverse effects such as neuropsychiatric manifestations, skin rash, hepatitis and dyslipidemia [1,3]. In clinical practice, concern over neuropsychiatric adverse effects often plays a role in the decision of whether or not to include efavirenz as part of ART. Prediction of therapeutic efficacy and the likelihood of developing psychiatric disorders have been associated with plasma efavirenz concentrations [4]. The preferable mid-dosing plasma level of efavirenz is 1000–4000 ng/ml to allow for optimized antiretroviral potency and to minimize the risk of neuropsychiatric toxicity. HIV-1-infected patients who receive standard-dose efavirenz and have plasma efavirenz concentration of <1000 ng/ml appear to have a higher risk for virological failure and emergence of selective drug resistance, while those with high plasma efavirenz concentrations of >4000 ng/ml may experience adverse CNS effects more frequently [4]. Many studies have highlighted the potential for serious psychiatric complications with efavirenz, including depression, psychosis, amnesia, extreme excitability, aggressive behavior, post-traumatic stress disorder symptoms and induced suicidal effect [3,4]. However, increased neuropsychiatric adverse effects were typically reported only during the first month after starting this medication [4–6]. Clinical trials have reported CNS side effects in >50% of patients following initiation of efavirenz-based ART. In patients initiating efavirenz therapy for the first time, the development of adverse effects may negatively influence adherence and subsequent treatment failure [6]. The effect of genetic polymorphisms on efavirenz pharmacokinetics is markedly considered because the plasma concentration of efavirenz has been found to be a reliable predictor of treatment failure and risk of neurologic side effects.

CYP2B6 polymorphisms, efavirenz concentrations & CNS adverse effects

Genetic variance among individuals influences the metabolism, distribution and elimination of drugs. Higher plasma efavirenz concentrations may be a result of genetic differences in the metabolism of this drug. Efavirenz is metabolized by CYP2B6, CYP2A6 and UGT2B7 [7]. However, CYP2B6 is the major metabolizing enzyme involved in the metabolism of efavirenz, and its genetic polymorphism is associated with increased plasma efavirenz concentration and a higher incidence of neurotoxicity during initial treatment [8]. The allelic variant 516G>T is associated with diminished activity of the CYP2B6 isoenzyme, increased plasma efavirenz concentrations and increased incidence of efavirenz-associated neuropsychological toxicity [4,5,7,9–11]. Gounden *et al.* found correlation between *CYP2B6* 516TT genotype and efavirenz concentrations, which resulted in increased incidence of fatigue, mood and sleep disorders after initiation of efavirenz [5]. Moreover, a previous study has established that *CYP2B6* T983C increases the predictive capability of *CYP2B6* G516T for efavirenz pharmacokinetics. Associations between increased plasma efavirenz exposure, *CYP2B6* 516G>T

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and 983T>C have been consistent across multiple studies and populations [12]. In addition, our studies of *CYP2B6* polymorphisms showed significant allelic variants (*CYP2B6* c.516G>T and c.785A>G polymorphisms), which may decrease the clearance of efavirenz by reducing the activity of the CYP2B6 enzyme and thereby increase plasma efavirenz concentration [9–11]. Prospective *CYP2B6* c.516G>T, c.785A>G and c.T983C genotyping has been proposed for identifying patients at risk of neurotoxicity for efavirenz-based ART in HIV-infected patients.

CYP2B6 polymorphisms & risk for treatment failure

Efavirenz has a low genetic barrier to HIV drug resistance. A single mutation, most frequently K103N in the reverse transcriptase gene, results in efavirenz resistance. The development of efavirenz resistance mutations may be due to repetitive exposure to subtherapeutic drug levels. Treatment failure has been found to be more frequent in patients with low efavirenz trough levels compared with those with high levels (>1100 ng/ml). As for efavirenz, some investigators have suggested that the lower limit for the therapeutic range of efavirenz should be raised from 1000 to 2300 ng/ml [13,14]. The median efavirenz concentration for patients with g.18492 heterozygous variants or homozygous variants was significantly lower than those with the wild-type genotype [Sukasem C, Manosuthi W, Koomdee N *et al.* Low efavirenz pharmacokinetics in HIV-1 infected Thai adults are associated with CYP2B6 polymorphism (2013), Submitted]. The information given by this SNP analysis may help to effectively identify HIV-infected individuals who might have a risk for treatment failure. Because the T allele in *CYP2B6* g.18492C>T has a high frequency among the HIV-infected population, its role as an indicator of clinical outcomes needs to be defined in this population.

Integrating *CYP2B6* pharmacogenetics in clinical practices

Efavirenz dose reduction or initiation of efavirenz treatment at reduced dose must be considered in *CYP2B6*6/*6* (516TT and 785GG) homozygotes, which could eliminate the problem of efavirenz-associated CNS symptoms. It may also decrease the risk of development of efavirenz resistance, an important issue in resource-limited countries. It is recommended to establish *CYP2B6* genotype in patients receiving efavirenz in order to predict their metabolizing behavior. Accordingly, to obtain efavirenz steady-state concentrations within the therapeutic range (1000–4000 ng/ml), it would be advisable to implement a gradual reduction in dose to 400 or 200 mg/day for patients that are intermediate or poor metabolizers, respectively [15].

Haas *et al.* reported on a patient with the *CYP2B6* 516T/T genotype who had chronic CNS symptoms and extremely high efavirenz concentration while receiving a 600-mg dose, but the symptoms were resolved by reducing the efavirenz dose to 200 mg [8]. Gatananga *et al.* showed that patients with the *CYP2B6* 516G>T SNP had significantly higher plasma efavirenz concentrations (>6000 ng/ml) on the standard dosing regimen. In that study, the reduction of the initial efavirenz dosages to either 400 or 200 mg resulted in the lowering of efavirenz concentrations towards the therapeutic range and an improvement in CNS-related symptoms in the majority of patients [15]. The HIV-1 load was successfully suppressed below the detection limit (50 copies/ml) at dosages that were reduced from 600 to 400 and 200 mg. Importantly, individuals who suffered from chronic CNS-related symptoms at the standard dosage showed an improvement with efavirenz dose reduction. Taken together, the quality of life of *CYP2B6* 516T/T genotype carriers who suffer from CNS-related symptoms can be improved by reducing efavirenz dose from the standard 600 to 400 or even 200 mg once daily [8,15].

> "*...the* CYP2B6 *18492 C>T genotype is associated with low plasma efavirenz concentrations, and may require a higher dose of efavirenz.*"

Therefore, a genotyping test for common functional variants of *CYP2B6*6*, which contains both the 516G>T and 785A>G polymorphisms, prior to the initiation of therapy is recommended for identifying patients at risk of efavirenz-associated neurotoxicity in clinical practice. Conversely, the *CYP2B6* 18492 C>T genotype is associated with low plasma efavirenz concentrations, and may require a higher dose of efavirenz. A cost–effectiveness study indicated that cost remains an issue for identifying *CYP2B6* 516 genotype. However, our laboratory (Laboratory for Pharmacogenomics, Ramathibodi Hospital, Thailand) has already developed a *CYP2B6* genotype detection system based on a PCR assay, which costs only approximately $67 per single test [9–11] [Sukasem C, Manosuthi W, Koomdee N *et al.* Low efavirenz pharmacokinetics in HIV-1 infected

Thai adults are associated with CYP2B6 polymorphism (2012) Submitted). Thus, the pharmacogenetics of *CYP2B6* may be used to guide efavirenz dosages. Additionally, genetic information about CYP2B6 may prove to be useful for the *a priori* dosing of efavirenz. Hence, *CYP2B6* genotyping should be introduced into routine clinical practice, where clinicians' decisions can be guided by the patient's genotype. Antiretroviral prescribing strategies could be improved by understanding whether certain individuals are genetically predisposed to CNS-related adverse effects or virological failure with efavirenz.

> "*In the future,* CYP2B6 *genotyping will likely move into clinical practice for HIV-infected patients treated with efavirenz and increasingly enable doctors to prescribe the right dosage of efavirenz for the first time for everyone.*"

In summary, pharmacogenetic testing of *CYP2B6* in HIV-infected patients offers evidence that this test can be used clinically to improve outcomes for patients receiving an efavirenz-based regimen. For this reason we suggest the testing of *CYP2B6* polymorphisms in routine clinical practice where the prevalence of the *CYP2B6* 516TT genotype is high. In the future, *CYP2B6* genotyping will likely move into clinical practice for HIV-infected patients treated with efavirenz and increasingly enable doctors to prescribe the right dosage of efavirenz for the first time for everyone. This would mean that patients will receive medicines that are safer and more effective, leading to better healthcare overall. However, this tool should not take the place of careful adherence counseling and monitoring, but rather should augment clinical practice.

Financial & competing interests disclosure

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Low efavirenz pharmacokinetics in HIV-1 infected Thai adults are associated with CYP2B6 polymorphism

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Abstract:	ABSTRACT Background The current study assessed the influence of the CYP2B6 genotype on efavirenz level. HIV-1 infections with plasma efavirenz concentration of <1,000 ng/mL appear to have great risk for emergence of drug resistance. Methods g.18492T>C (rs2279345) polymorphism at the gene encoding the CYP2B6 in 139 HIV-infected Thai adults were genotyped. Plasma efavirenz concentrations at 12 hours after dosing were measured using a validated high performance liquid chromatopraphy. Relationship between plasma efavirenz concentrations and g.18492T>C polymorphims were analysed. Results The frequency of g.18492T>C heterozygous (T/C) and homozygous mutant (C/C) was 39% and 7%, respectively. In the entire cohort, the median efavirenz plasma concentration was 2,260 ng/mL (IQR 1,442-3,665). Plasma efavirenz concentration for patients with g.18492 CC (1,200 ng/mL, IQR 1,070-1,820) or TC (1900 ng/mL, IQR 1,340-2,290) genotype were significantly lower than those with homozygous wild-type (3,210 ng/mL, IQR 1,903-5,293), p-value <0.001. Conclusions The CYP2B6 g.18492T>C polymorphism was significantly associated with lower efavirenz concentrations than those with homozygous wild-type in HIV-1 infections. Genetic polymorphism of CYP2B6 which associated to low plasma efavirenz

	level may have usefulness for optimized efavirenz dose which used in HIV-1 infe patient. Further studies in the clinical setting will need to be conducted before such approach can be recommended for widespread use.
Author Comments:	

June, 30, 2013

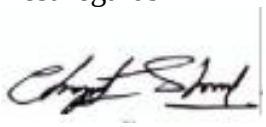
Dear the Editor
INFECTION

Enclosed is our manuscript on the title of "Low efavirenz pharmacokinetics in HIV-1 infected Thai adults are associated with CYP2B6 polymorphism" submission.

The results presented in this paper have not been submitted or accepted in whole or part for publication elsewhere. The all authors have seen and approved the content and have contributed significantly work for this manuscripts. Additionally, this study has no conflict of interest.

It would be appreciated if you please consider publishing this study in the **INFECTION.**

Best regards



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Low efavirenz pharmacokinetics in HIV-1 infected Thai adults are associated with CYP2B6 polymorphism

Running headline: Low plasma efavirenz level and SNP *g.18492T>C*

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ABSTRACT

Background The current study assessed the influence of the *CYP2B6* genotype on efavirenz level. HIV-1 infections with plasma efavirenz concentration of <1,000 ng/mL appear to have great risk for emergence of drug resistance.

Methods *g.18492T>C* (rs2279345) polymorphism at the gene encoding the CYP2B6 in 139 HIV-infected Thai adults were genotyped. Plasma efavirenz concentrations at 12 hours after dosing were measured using a validated high performance liquid chromatopraphy. Relationship between plasma efavirenz concentrations and *g.18492T>C* polymorphims were analysed.

Results The frequency of *g.18492T>C* heterozygous (T/C) and homozygous mutant (C/C) was 39% and 7%, respectively. In the entire cohort, the median efavirenz plasma concentration was 2,260 ng/mL (IQR 1,442-3,665). Plasma efavirenz concentration for patients with g.18492 CC (1,200 ng/mL, IQR 1,070-1,820) or TC (1900 ng/mL, IQR 1,340-2,290) genotype were significantly lower than those with homozygous wild-type (3,210 ng/mL, IQR 1,903-5,293), p-value <0.001.

Conclusions The *CYP2B6 g.18492T>C* polymorphism was significantly associated with lower efavirenz concentrations than those with homozygous wild-type in HIV-1 infections. Genetic polymorphism of *CYP2B6* which associated to low plasma efavirenz level may have usefulness for optimized efavirenz dose which used in HIV-1 infected patient. Further studies in the clinical setting will need to be conducted before such an approach can be recommended for widespread use.

Key words: HIV, *CYP2B6*, efavirenz, *g.18492T>C*, subtherapeutic level, Thai

INTRODUCTION

Efavirenz is a potent and effective non-nucleoside reverse transcriptase inhibitor (NNRTI) that is commonly used in initial therapy as part of the first-line antiretroviral therapy (ART) for HIV-1 infected individuals in Thailand [1,2]. Such a country, NNRTI-based regimen has been normally prescribed, because of its availability, and the low cost. The high prevalence of HIV drug resistance in Thai HIV-1 infected patients was observed [3-6].

Many studies reported the high inter-individual variability of efavirenz plasma levels in HIV-1 patient involve the attainment of a long-term benefit with efavirenz treatment [7-9]. The preferable mid-dosing plasma level of efavirenz is 1,000–4,000 ng/mL to optimise antiretroviral potency and to minimise the risk for neuropsychiatric toxicity [10-14] . HIV-1 patients who receive treatment with efavirenz-based regimen and have plasma efavirenz concentration of <1,000 ng/mL seem to have a higher risk for virological failure [14-17] and emergence of selective drug resistance, while patients with efavirenz concentration > 4,000 ng/mL may experience adverse effect of central nervous system [10-20].

CYP2B6 is the major enzyme which responsible for the metabolism and clearance of efavirenz [7-9, 12]. In fact, the *CYP2B6* gene has several polymorphisms and this may influence the isoenzyme activity. The effect of genetic polymorphisms on efavirenz pharmacokinetics retains much attention because the plasma concentration of efavirenz has been found to be a predictor of treatment failure and risk of adverse drug reaction (ADR) including headache, dizziness and insomnia [10-16]. Previous studies showed the significant allelic variant (*CYP2B6 c.516G>T* and c.*785A>G* polymorphisms) which may decrease the clearance of efavirenz by reduce the activity of CYP2B6 enzyme, increase efavirenz plasma concentration [7, 8]. In contrast, virological failure was reported in 50% of patients with low mid-dosing efavirenz levels (< 1,000 ng/mL) [14].

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To date, data of *CYP2B6* polymorphism that may be associated with low efavirenz level is very limited. This study therefore was to examined the frequency of *CYP2B6 g.18492T>C* polymorphism including the impact of this polymorphism on efavirenz plasma concentration in HIV-1 infected Thai patients receiving efavirenz for treatment since these patients may have subtherapeutic level of efavirenz and subsequent treatment failure.

MATERIALS AND METHODS

Subjects

All subjects provided written informed consent within the cohort study, which included the use of stored samples for future research following specific ethical clearance. Following written inform consent form, 139 Thai adults with HIV-1 infection were recruited at Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand. Enrolled patients were aged ≥ 18 years, had no opportunistic infection and taking antiretroviral regimen with NNRTI-based regimen. All sample were submitted to Laboratory for Pharmacogenomics and Personalized Medicine, Ramathobidi Hospital, Mahidol University for *CYP2B6 g.18492T>C* (rs2279345) genotype. The other 7 SNPs (c.64C>T, c.499C>G, c.516G>T, c.785A>G, c.1375A>G, c.1459C>T, and g.21563C>T) had been excluded and only the patients who carried CYP2B6 haplotype *1/*1 were analysed.

Of all, a total of 100 HIV-1 infections were further analyzed for the relationship between plasma efavirenz concentration and genetic polymorphism in this study. All patients were receiving tenofovir (300 mg), lamivudine (300 mg) and efavirenz (600 mg) at bed time. Mid-dose efavirenz plasma concentration was determined at 12 weeks following initiation of antiretroviral therapy. Patients receiving concomitant treatments that could potentially affect efavirenz pharmacokinetics were excluded. This study was approved by the Ethics Committee of the Faculty of Medicine Ramathibodi Hospital, Bangkok, Thailand.

CYP2B6 Genotyping

CYP2B6 g.18492T>C (rs2279345) identified using HapMap (www.hapmap.org) data on Japanese and Han Chinese populations with an r2 > 0.8 were assessment [7]. Pre-designed TaqMan assays (Applied Biosystems, Foster City, CA), were used to genotype the *CYP2B6 g.18492T>C* (assay ID C__26823975_10). For SNP analysis, this SNP were genotyped using allele-specific fluorogenic 5’ nuclease chain reaction assay with predesigned primers and

TaqMan MGB probes (TaqMan SNP Genotyping Assay; Applied Biosystems, Foster City, CA).Sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest used two TaqMan MGB probes; one probe was labeled with VIC dye and detected the Allele 1 sequence, the second probe was labeled with FAM dye and detected the Allele 2 sequence.

Measurement of efavirenz plasma concentrations

Fasting plasma efavirenz level at 12 hours after dosing was measured using a validated high performance liquid chromatopraphy assay at 12 weeks of ART inititation. Briefly, patient plasma samples (300 µL) and all calibration and control samples were heat inactivated in a water bath at 56°C for 30 minutes prior to assay. Sample pretreatment involved protein precipitation with acetonitrile (360 µL) and following centrifugation the sample supernatant was injected into the HPLC machine. Chromatography was performed using an Agilent 1100 HPLC machine with an Omnispher C18 (150 x 4.6 mm I.D/particle size 5 µm) analytical column (Varian, CA, USA), a Chromquard RP guard column and a mobile phase consisting of 10 mM KH2PO4 pH 3.1- acetonitrile (50:50, v/v). This assay was developed at the Department of Clinical Pharmacology at the University Medical Centre Nijmegen, The Netherlands. The sample peak heights were processed by ChromQuest Software version 4.1.

Statistical Analysis

Genotype distributions were tested for Hardy-Weinberg equilibrium using exact tests. SNPs with a call rate < 95% were omitted. A Kruskal-Wallis test was used to test for significant difference in efavirenz concentrations between *CYP2B6 g.18492T>C* (rs2279345). Mann-Whitney U tests were used to compare efavirenz concentrations between two genotypes. Statistical significance was defined as $P<0.05$.

RESULTS

Subjects and clinical characteristics

A total of 139 HIV-infected Thai individuals initiated therapy with NNRTI-based regimen were enrolled in this study. All infections were Thai, and their mean of body weight±SD was 54 ± 10. Among them, 108 (78.0%) and 31 (22.0%) of 139 subjects were males and females, respectively. The median (IQR) CD4 T-lymphocyte counts was 42 (17-105) cells/mm^3 and median (IQR) plasma HIV-1 RNA was 5.8 (5.4-6.3) log copies/mL. Among theses patients, liver and renal function tests were within normal ranges. Demographics and laboratory data of 139 patients are summarized in table 1.

Allele frequency of *CYP2B6 g.18492T>C*

All 139 subjects were succesfully performed by TaqMan genotyping assay for determined *CYP2B6 g.18492T>C* which was found to be in Hardy–Weinberg equilibrium (P>0.05). The frequencies of *CYP2B6* polymorphism (*g.18492T>C*) is summarized in Table 2. The analysis of the *g.18492T>C* genetic polymorphism in the study population revealed that 54% of patients had the wild-type genotype (T/T), 39% had the heterozygous genotype (C/T), and 7% had the polymorphic homozygous genotype (C/C). The minor allele frequency was 0.265.

Correlation between *g.18492T>C* and plasma efavirenz concentration

Overall, the median plasma efavirenz concentration was 2,260 ng/mL (IQR 1,442-3,665) and had a high interindividual variability ranging from 580 ng/mL to 23,350 ng/mL. The lowest efavirenz concentration was observed in subject with homozygous genotype (C/C), and the highest concentration was found in subject with wild-type alleles (T/T).

As shown in figure 1, the median efavirenz concentration were as follows: 3,210 ng/mL (IQR 1,903-5,293 ng/mL) for the T/T genotype; 1,900 ng/mL (IQR 1,340-2,290 ng/mL) for the T/C genotype; and 1,200 ng/mL (IQR, 1,070-1,820 ng/mL) for the C/C

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genotype. Although, the majority of the patients taking efavirenz had a concentration above 1000 ng/mL, the median efavirenza level for patients with the *g.18492* heterozygous variants or homozygous variants was significantly lower than those with the wild-type genotype (P<0.001).

DISCUSSION

Experience to subtherapeutic level of antiretroviral drugs is one of the main causes of drug resistance and eventually treatment failure. HIV-resistant strains are definitely selected when the drugs do not accomplish the therapeutic level [21-23]. NNRTI-based regimens are recommended as preferred first-line treatment [1, 24]. Meanwhile efavirenz has been used widely to treatment HIV-infection in developing countries, pharmacogenetics study of *CYP2B6* will be extreme importance. The association between *CYP2B6 g.18492T>C* and efavirenz plasma concentration is largely unknown [7, 25].

This study was aimed to determine the frequency of *CYP2B6 g.18492T>C* in Thai HIV-1 infections. Moreover, the association between steady-state plasma efavirenz level and *g.18492T>C* polymorphism in *CYP2B6* was also investigated. In this study, we found that *CYP2B6 g.18492C>T* was less frequent in Thai HIV-1 infections (0.27) than in the HapMap (http://hapmap.ncbi.nlm.nih.gov/) European population (0.40), but was found to be silimilar to those of the HapMap Chinese (0.30), Japanese (0.27) and Sub Saharan African (0.258) population. Therefore, significant diffrence was observed between Thai population and the Caucasian population.

The impact of *CYP2B6 g.18492T>C* polymorphism on steady-state efavirenz plasma concentrations could be demonstrated in this study. In agreement with previous studies, the median efavirenza concentration for patients with the *g.18492* heterozygous variants or homozygous variants was significantly lower than those with the wild-type genotype [7]. In this study, we confirm that homozygous mutant of *CYP2B6 g.18492T>C* may increase CYP2B6 activity and, as a consequence, decreases plasma efavirenz concentration. A previous study showed that a 1,000 to 4,000 ng/mL range of plasma efavirenz concentration at a middosing interval is a suitable target for dose individualization, whereas treatment failure is associated with low efavirenz plasma levels [10-14]. Consequently, this can result

in risk of subtherapeutic efavirenz plasma concentrations and favor the development of viral resistance against efavirenz [14]. In the meantime, clinical implications may be derived from this study. The information given by this single-SNP analysis may help to easily identify HIV-infection who have a risk for treatment failure.

Efavirenz has a low genetic barrier to viral drug resisitance, so that a single mutation, most frequently K103N in the reverse transcriptase gene [3-6, 26]. The development of efavirenz-resistant mutant is probable to be facilitated by repetitive exposure to subtherapeutic drug levels. Treatment failure has been found to be more frequent in patients with low efavirenz trough level, compared with those with high level (>1100 ng/mL) [27, 28]. As for efavirenz, some investigators have suggested that the lower limit for the therapeutic range of efavirenz should be raised from 1,000 ng/mL to 2,300 ng/mL [29]. The patients who present of *g.18492T>C* variants may be close to subtherapeutic drug concentration. Furthermore, Haplotype analysis of *CYP2B6* at 5 loci found that the TGATC haplotype (*3003T>C, 516G>T, 785A>G, g.18492T>C and 21563C>T*) was significantly associated with nevirapine concentration with increased clearance and lower exposure in Thai HIV infections [8]. Thus, the association between low efavirenz concentration and efavirenz treatment failure urgently needs studies in Thai HIV-infected population.

Therapeutic drug monitoring of antiretrovirals has been incorporated as part of the diagnostics for HIV-infected patients in the national guidelines in many of countries [30]. Thus, pharmacogenetics and pharmacokinetics combined with therapeutic drug monitoring should be used to guide efavirenz dosages. Additionally, genetic information of *CYP2B6* may prove to be useful for the a priori dosing of efavirenz and nevirapine [7-9]. Hence, *CYP2B6* genotyping should be introduced into routine clinical practice, clinicians should initially be guided by the genotype and phenotype assessed through the plasma efavirenz concentrations obtained in therapeutic drug monitoring.

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In conclusion, results from this and previous study [7] suggest that *CYP2B6* T-to-C polymorphism at position 18492 was strongly associated with lower efavirenz plasma levels. Because the T allele in *CYP2B6 g.18492T>C* had high frequencies among Thai HIV-infected population, their role as an indicator of clinical outcomes needs to be defined in this population and may have a global impact on HIV/AIDS treatment with the increasing used efavirenz in developing countries. In order to confirm these preliminary results quantifying the influence of genetic factors, further prospective studies with larger data sets should be carried out.

Acknowledgments

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Table1: Relationship between *CYP2B6* polymorphisms and efavirenz plasma concentrations.

Genetic polymorphisms	n (%) n=100	Minor Allele Frequency	EFV plasma concentration (mg/L), Median (IQR)
CYP2B6 g.18492C>T(rs2279345)			
TT	54 (0.54)		3.210 (1.903-5.293)
TC	39 (0.39)	T= 0.265	1.900 (1.340-2.290)
CC	7 (0.07)		1.200 (1.070-1.820)
P-value			0.000*

* Statistical significant was indicated by a Kruskal-Wallis test.

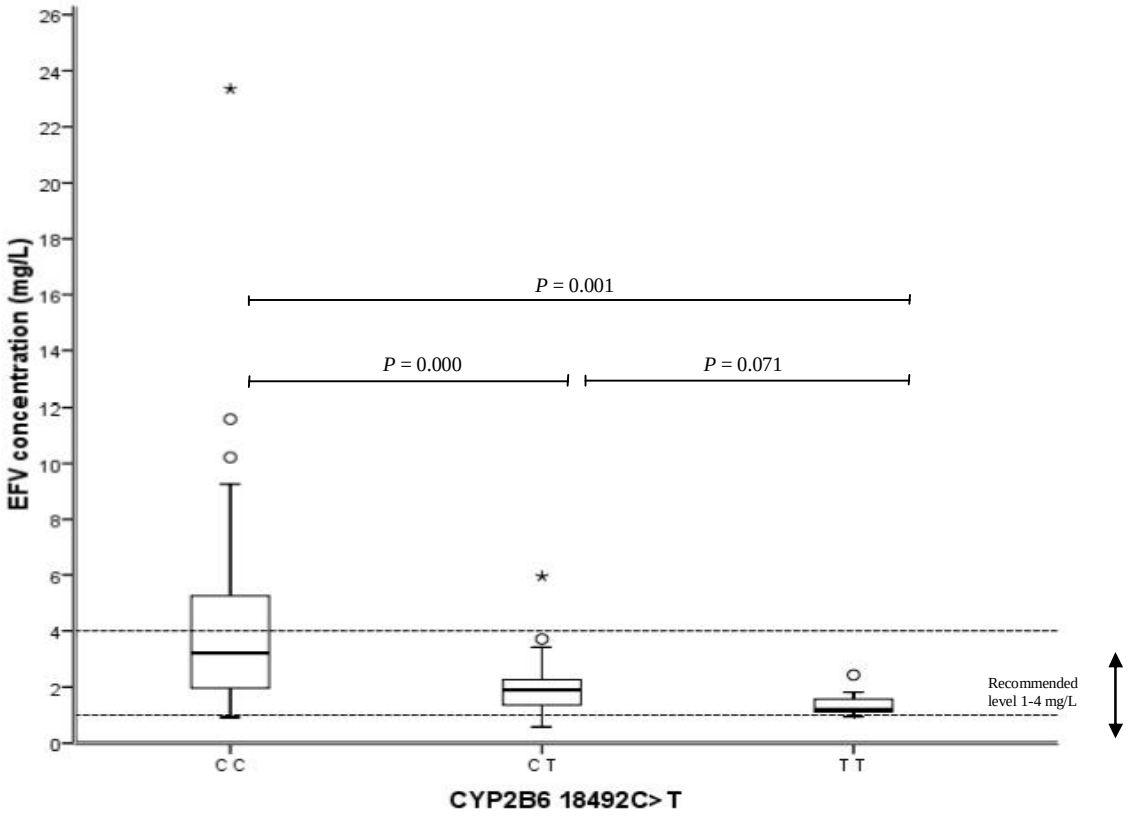
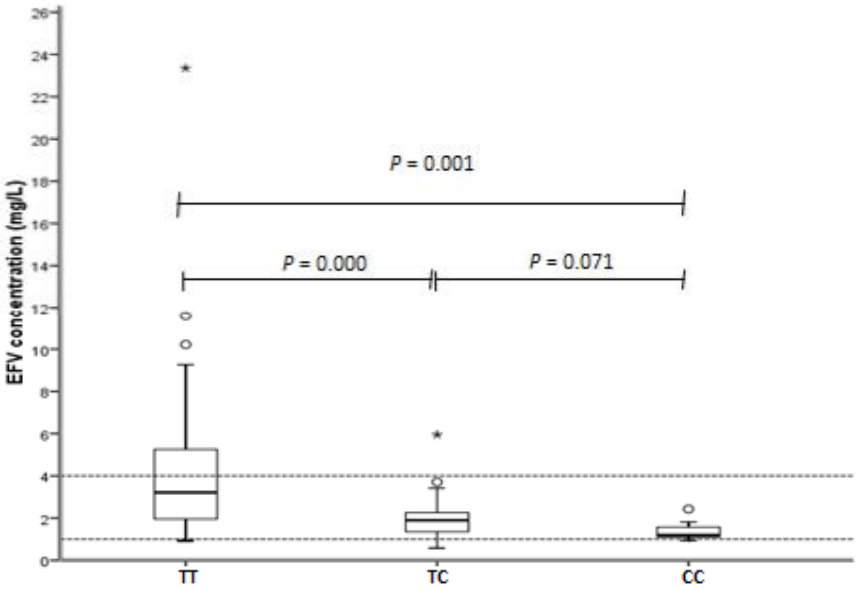


Figure 1. single nucleotide polymorphisms of CYP2B6 and plasma efavirenz levels at 12 hours after dosing. Middle bar indicated the median, upper and lower bars indicate 25th and 75th interquartile ranges. The medians and 25th and 75th interquartile ranges were displayed on each box. P values were shown only significant differences between genotypes.



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Antimicrobial Agents and Chemotherapy

Impact of Pharmacogenetic Markers of *CYP2B6*, Clinical Factors, and Drug-Drug Interaction on Efavirenz Concentrations in HIV/Tuberculosis-Coinfected Patients

Weerawat Manosuthi, Chonlaphat Sukasem, Aroon Lueangniyomkul, Wiroj Mankatitham, Supeda Thongyen, Samruay Nilkamhang, Sukanya Manosuthi and Somnuek Sungkanuparph

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Impact of Pharmacogenetic Markers of *CYP2B6*, Clinical Factors, and Drug-Drug Interaction on Efavirenz Concentrations in HIV/Tuberculosis-Coinfected Patients

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Comprehensive information on the effects of cytochrome P450 2B6 (*CYP2B6*) polymorphisms, clinical factors, and drug-drug interactions on efavirenz concentrations in HIV/tuberculosis-coinfected (HIV/TB) patients is unavailable. A total of 139 HIV/TB adults, 101 of whom received a rifampin-containing anti-TB regimen, were prospectively enrolled to receive efavirenz (600 mg)/tenofovir/lamivudine. Nine single nucleotide polymorphisms (SNPs) within *CYP2B6* were genotyped. Plasma efavirenz concentrations were measured at 12 weeks. The median (interquartile range [IQR]) efavirenz concentration was 2.3 (1.4 to 3.9) mg/liter. The SNPs (frequencies of heterozygous and homozygous mutants) were 64C>T (10% and 1%), 499C>G (0% and 0%), 516G>T (47% and 8%), 785A>G (54% and 10%), 1375A>G (0% and 0%), 1459C>T (3% and 0%), 3003C>T (44% and 27%), 18492T>C (39% and 6%), and 21563C>T (57% and 5%). The four most frequent *CYP2B6* haplotypes identified were *1/*6 (41%), *1/*1 (35%), *1/*2 (7%), and *6/*6 (7%). The heterozygous/homozygous mutation associated with low efavirenz concentrations was 18492T>C ($P < 0.001$), and those associated with high efavirenz concentrations were 516G>T, 785A>G, and 21563C>T (all $P < 0.05$). Haplotype *1/*1 was associated with low efavirenz concentrations, and *6/*6, *1/*6, and *5/6 were associated with high efavirenz concentrations. As shown by multivariate analysis, low efavirenz concentrations were significantly associated with the *1/*1 haplotype (beta = −1.084, $P = 0.027$) and high body weight (beta = −0.076, $P = 0.002$). In conclusion, pharmacogenetic markers of *CYP2B6* have the greatest impact with respect to inducing low plasma efavirenz concentrations in HIV/TB Thai patients.

In the resource-limited countries, patients with human immunodeficiency virus (HIV) infection often present late with advanced acquired immune deficiency syndrome (AIDS) and major opportunistic infections, of which tuberculosis (TB) is one of the most common (1–4). A rifamycin-containing antituberculosis regimen is essential in treatment of tuberculosis. Rifampin is a potent hepatic cytochrome P450 enzyme inducer, leading to accelerated drug clearance and a significant reduction in plasma concentrations of particular antiretroviral drugs (5). Efavirenz is a nonnucleoside reverse transcriptase inhibitor (NNRTI) which is mainly metabolized by hepatic cytochrome P450 2B6 (*CYP2B6*). Coadministration of efavirenz and two nucleoside reverse transcriptase inhibitors (NRTIs) with rifampin is currently recommended as a preferred antiretroviral regimen in treating patients who are coinfected with HIV and *Mycobacterium tuberculosis*, particularly where rifabutin is not available (6).

The range of acceptable plasma concentrations of efavirenz at 12 h is currently proposed to be 1 to 4 mg/liter (6, 7). Subtherapeutic efavirenz concentrations occur when efavirenz is coadministered with rifampin, leading to subsequent failure of treatment with an efavirenz-based regimen (7, 8). In contrast, concentrations above the therapeutic range increase the risk of drug-related toxicity outcomes, such as neuropsychiatric side effects (7). The *CYP2B6* gene is highly polymorphic (9, 10). Most previous studies have shown *CYP2B6* single nucleotide polymorphisms (SNPs), particularly 516G>T, to be associated with high plasma concentrations of efavirenz and its drug-related toxicity (11–13). To date, data concerning these polymorphisms incorporated with clinical factors and drug-drug interactions that may be associated with low efavirenz concentrations are very limited. In addition, the differences in plasma efavirenz concentrations among previous published data may reflect different *CYP2B6* SNPs and haplotypes. This study was therefore conducted to examine the impact of nine SNPs and haplotypes of the *CYP2B6* enzyme, potential clinical factors, and drug-drug interactions on plasma efavirenz concentrations in Thai patients coinfected with HIV and tuberculosis, since these patients may have a subtherapeutic concentration of efavirenz and subsequent treatment failure.

(Part of this research was presented at the 52nd Interscience Conference of Antimicrobial Agents and Chemotherapy [ICAAC], San Francisco, CA, 2012 [poster round] [14].)

MATERIALS AND METHODS

Patients coinfected with HIV and tuberculosis were prospectively enrolled between October 2009 and May 2011 at the Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand. The institutional ethics committees of the Bamrasnaradura Infectious Diseases Institute and the Thai Ministry of Public Health approved the study. All patients provided written, informed consent prior to enrollment.

Patients were categorized according to antituberculosis regimen, rifampin-containing regimen, and other regimens without rifampin. They were followed through 12 weeks after initiation of antiretroviral therapy

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(ART). The objectives were to study the frequencies of nine *CYP2B6* polymorphisms in HIV-infected Thai adults and to identify SNPs, haplotypes, and clinical factors that are associated with plasma efavirenz concentrations at 12 h after dosing. Inclusion criteria were as follows: (i) HIV-infected individuals 18 to 60 years of age, (ii) newly clinically diagnosed active tuberculosis, positive acid-fast staining, or a positive culture for *Mycobacterium tuberculosis*, (iii) treatment with an antituberculosis regimen 4 to 12 weeks prior to enrollment, (iv) naïveté to ART, (v) baseline CD4 cell count < 350 cells/mm^3, and (vi) participation and informed consent. Exclusion criteria were as follows: (i) inability to tolerate efavirenz due to any reason, (ii) serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) levels > 5 times the upper limit of the normal range, (iii) a serum creatinine level > 2 times the upper limit of the normal range, (iv) treatment with a medication that has drug-drug interactions with efavirenz or rifampin, (v) treatment with immunosuppressive drugs, and (vi) pregnancy or lactation.

All patients were started on a once-daily antiretroviral regimen of tenofovir (300 mg), lamivudine (300 mg), and efavirenz (600 mg) at bedtime irrespective of baseline patient body weight. ART was initiated at between 4 weeks and 12 weeks of TB treatment initiation. The dosage of rifampin was 450 mg/day for body weight ≤ 50 kg and 600 mg/day for body weight > 50 kg. The antituberculosis regimen was isoniazid, rifampin, pyrazinamide, and ethambutol for the first 2 months followed by isoniazid and rifampin for the subsequent 4 to 7 months. Patients who received other antituberculosis regimens without rifampin were those who initially could not tolerate rifampin due to adverse effects or hypersensitivity. The patients had follow-up visits at week 2, week 6, and week 12 after initiation of ART, when they were assessed clinically and/or blood samples were taken. Adherence counseling was given to the patients, and adherence to treatment was assessed with a questionnaire. Any patient with adherence of less than 80% was excluded from the analysis. In addition, all patients were instructed to take their medication regularly at least 2 weeks prior to blood collection.

The fasting plasma efavirenz concentration at 12 h after dosing (for patients observed taking dosing) was measured using a validated high-performance liquid chromatography assay at 12 weeks of ART initiation. This assay was developed at the Department of Clinical Pharmacology at the University Medical Centre Nijmegen, Nijmegen, The Netherlands. The sample peak heights were processed by ChromQuest software version 4.1. CD4 cell counts were determined by flow cytometry using monoclonal antibodies with three-color reagent (TriTEST; Becton, Dickinson BioSciences) and analyzed using a FACScan flow cytometer (Becton, Dickinson BioSciences). Plasma HIV-1 RNA levels determined by real-time PCR using a Cobas AmpliPrep/Cobas TaqMan HIV-1 test (Roche Molecular Systems Inc., Branchburg, NJ) and alanine aminotransferase (ALT) enzyme levels were assessed at week 0 and at week 12 after ART.

At week 0 of ART, DNA was isolated from the stored EDTA cell pellets using a QlAamp DNA blood minikit (Qiagen, Hilden, Germany). Genomic DNA was quantified by a ND-1000 UV spectrophotometer (NanoDrop Technologies, Wilmington, DE) at 260 nm. A total of nine SNPs within *CYP2B6* were genotyped. SNPs 516G>T and 785A>G have been previously reported to influence plasma efavirenz concentrations (15), and three *CYP2B6* SNPs, 3003T>C, 18492C>T, and 21563C>T, were identified using the International Haplotype Mapping Project (HapMap) (http://www.hapmap.org) in Japanese and Han Chinese subjects. SNP499C>G was associated with high plasma efavirenz concentrations in Japanese subjects, and the remaining three SNPs, i.e., 64C>T, 1375A>G, and 1459C>T, were reported in Chinese subjects (16). The *CYP2B6* haplotype determination was interpreted using The Human Cytochrome P450 (*CYP*) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2b6.htm). All SNPs were included for *CYP2B6* haplotype interpretation except 3003T>C and 18492C>T.

All analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL). Frequencies (percentages), means ± standard deviations (SD), and medians (IQR at the 25th and 75th percentiles) were used to describe patients' characteristics and laboratory parameters. The independent variables were evaluated with simple linear regression analysis to identify the factors that were associated with plasma efavirenz concentration. Any independent variable with a *P* value of less than 0.1 was included in the model of multiple regression analysis. Possible predictive factors for plasma efavirenz concentrations were evaluated with a linear regression model by adjusting for confounding factors, i.e., body weight at week 12, receiving rifampin, having positive hepatitis C virus antibody (anti-HCV) results, and haplotype. The factors of receiving rifampin, having anti-HCV, and haplotype were examined as dichotomous variables, and the remaining factors were examined as continuous variables. The beta values were estimated. The Pearson's correlations were used to study the relationships between the plasma efavirenz concentration and patient body weight. Interpatient variability of plasma efavirenz concentration was expressed as percent coefficient of variation (CV).

TABLE 1 Baseline characteristics of 139 patients coinfected with HIV and *M. tuberculosis*

Characteristic	Values (n = 139)
Demographics	
No. (%) of males	108 (78)
Mean age (yr) ± SD	37 ± 8
Mean body wt (kg) ± SD	54 ± 10
No. (%) of patients with indicated site of TB	
Lung	64 (46)
Cervical lymph node	14 (10)
Disseminated TB	56 (40)
Meninges	3 (3)
Colon	2 (1)
Laboratory parameters	
Median (IQR) CD4 cell count/mm^3	42 (17–105)
Median (IQR) % CD4 cells	6 (3–11)
Median (IQR) log plasma HIV-1 RNA copies/ml	5.8 (5.4–6.3)
Median (IQR) g hemoglobin/dl ± SD	10.8 (9.5–11.9)
Median (IQR) mg serum alkaline phosphatase/dl ± SD	105 (73–170)
Median (IQR) U alanine aminotransferase/liter ± SD	30 (19–46)
Median (IQR) mg albumin/dl ± SD	3.4 (3.0–3.8)
Median (IQR) mg total bilirubin/dl ± SD	0.41 (0.31–0.71)
Median (IQR) mg serum creatinine/dl ± SD	0.7 (0.6–0.8)
No. (%) of hepatitis B virus antigen-positive results	6 (4)
No. (%) of hepatitis C antibody-positive results	18 (13)

RESULTS

A total of 150 patients were initially enrolled and started ART. Eight patients discontinued efavirenz due to adverse events prior to measurement of the plasma efavirenz concentration, and three patients were excluded due to poor adherence. Demographic features and baseline laboratory parameters of the remaining 139 patients are shown in Table 1. The median (IQR) CD4 cell count was 42 (range, 17 to 105) cells/mm^3, and the median (IQR) plasma HIV-1 RNA level was 5.8 (5.4 to 6.3) log copies/ml. Of 139 patients, 38 received antituberculosis regimens without rifampin. At week 12, the median (IQR) plasma efavirenz concentration for all 139 patients was 2.3 (1.4 to 3.9) mg/dl. Figure 1 shows the frequencies of each of the SNPs and the box plots of plasma efavirenz concentrations for nine *CYP2B6* SNPs. The three most frequent *CYP2B6* SNPs detected were 3003C>T, 785A>G, and 21563C>T. Nucleotide substitutions were not detected at posi-

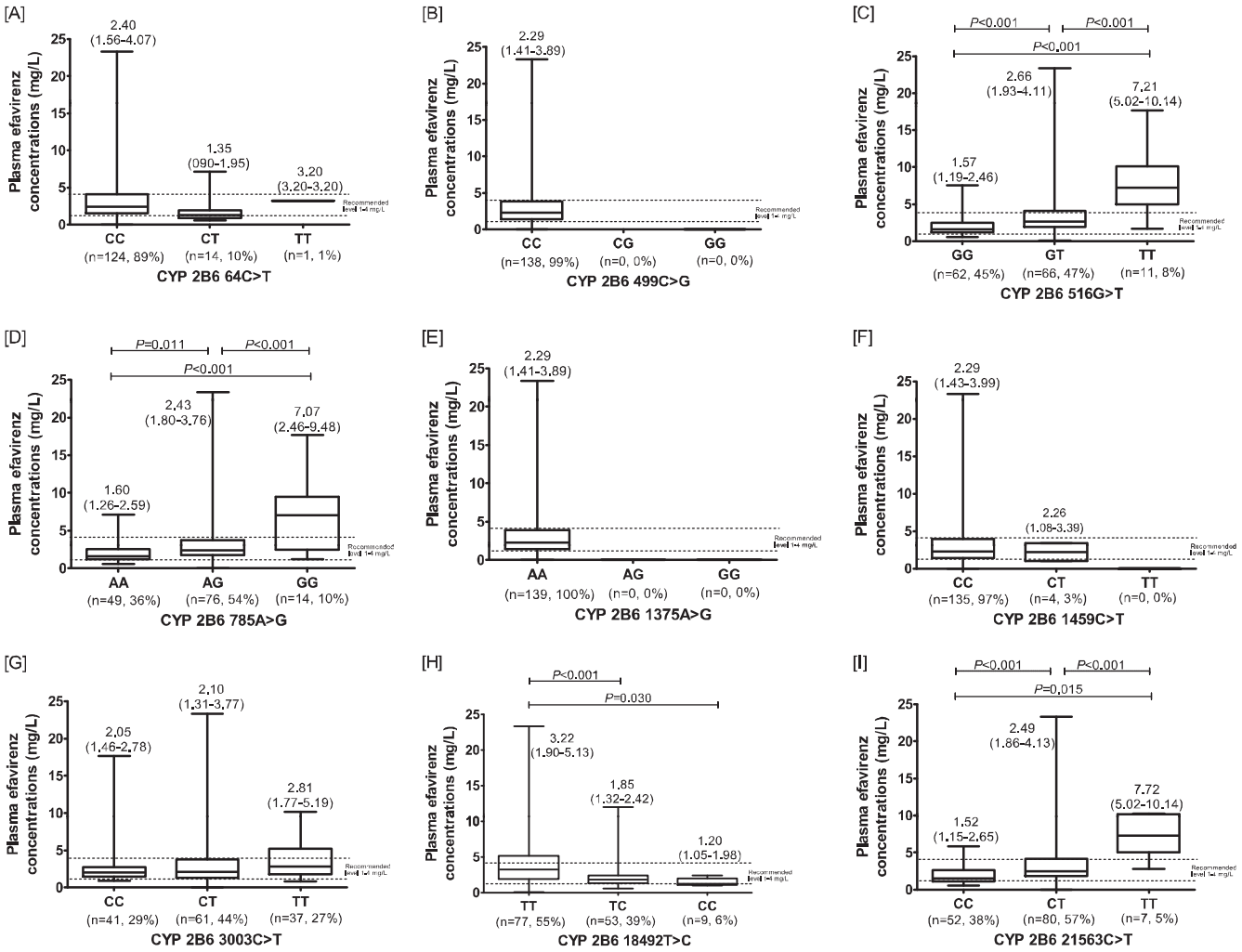

FIG 1 Nine single nucleotide polymorphisms of *CYP2B6* and plasma efavirenz concentrations at 12 h after dosing. The middle bar indicates the median, and the upper and lower bars indicate the 25th and 75th interquartile ranges. The medians and 25th and 75th interquartile ranges are displayed on each box. *P* values are shown only for significant differences between genotypes.

tions 499 and 1375. Three *CYP2B6* SNPs, including 516G>T, 785A>G, and 21563C>T, were found to be associated with high plasma efavirenz concentrations, but 18492T>C was associated with a low plasma efavirenz concentration. Figure 2 shows the frequencies of *CYP2B6* haplotypes and plasma efavirenz concentrations by haplotype. Frequent *CYP2B6* haplotypes were *1/*6 (41%), *1/*1 (35%), *1/*2 (7%), *6/*6 (7%), *4/*6 (4%), *1/*4 (3%), *2/*4 (1%), *2/*6 (1%), and *5/*6 (1%). Three of 9 haplotypes identified, including *6/*6, *1/*6, and *5/*6, were associated with high plasma efavirenz concentrations. There was no *CYP2B6* genetic mutation in 35% of all patients for whom the haplotype was determined to be *1/*1.

Univariate and multivariate analysis of possible factors associated with the plasma efavirenz concentration is shown in Table 2. By multivariate analysis, factors associated with a low plasma efavirenz concentration included specific haplotype and high body weight ($P < 0.05$), but the factor "receiving rifampin" did not reach a significant level. Figure 3 displays the relationship between body weight at week 12 and the plasma efavirenz concentration by haplotype. By correlation analysis, there appears to be a relationship between high body weight and a low plasma efavirenz concentration ($P < 0.05$). Median (IQR) plasma efavirenz concentrations for all patients, 101 patients who were concurrently receiving efavirenz and rifampin, and 38 patients who did not receive rifampin were 2.3 (1.4 to 3.9) mg/dl, 2.1 (1.3 to 3.5) mg/dl, and 2.7 (1.8 to 5.4) mg/dl, respectively. The interpatient variabilities of plasma efavirenz concentrations in the corresponding groups were 95%, 75%, and 107%, respectively. Seven of 101 (7%) of the patients who were concurrently receiving rifampin and 3 of 38 (8%) of those who were not concurrently receiving rifampin had a plasma efavirenz concentration below the recommended concentration.

DISCUSSION

The emergence of HIV drug resistance is likely facilitated by prolonged exposure to a subtherapeutic concentration of antiretroviral drugs. Therefore, maintaining an adequate drug concentration is very important for achieving long-term virologic suppression in the treatment of HIV infection. To date, the utility of phamacogenetic markers to predict chance of antiretroviral failure has been limited. This is the first study aimed at examining the impact of

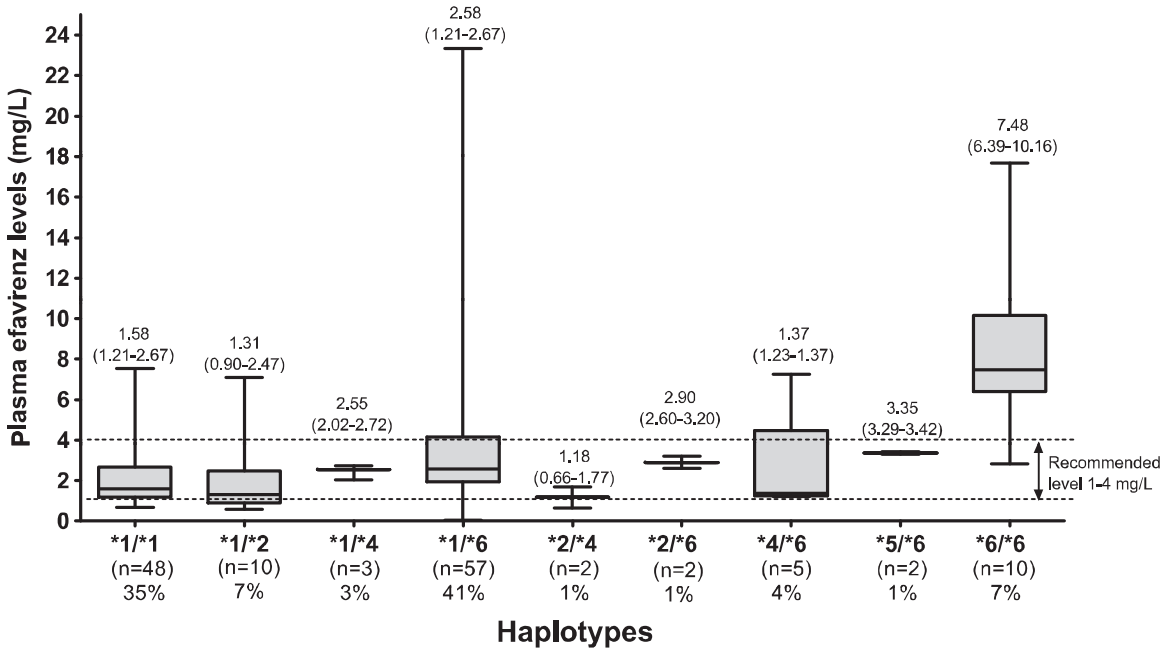

FIG 2 Distributions of *CYP2B6* haplotypes and plasma efavirenz concentrations at 12 h after dosing by haplotypes. The middle bar indicates the median, and the upper and lower bars indicate the 25th and 75th interquartile ranges. The medians and 25th and 75th interquartile ranges are displayed on each box.

TABLE 2 Univariate and multivariate analysis of plasma efavirenz concentration as the dependent variable

Parameter	Univariate analysis		Multivariate analysis[a]	
	P	Beta	*P*	Beta
Body wt at wk 12	0.001	−0.086	0.002	−0.076
Male gender	0.916	−0.007		
Age	0.221	0.040		
Baseline serum creatinine	0.487	−0.989		
Serum ALT	0.498	0.007		
Hepatitis B virus antigen positive	0.787	−0.354		
Hepatitis C antibody positive	0.033	1.678	0.332	0.674
Patients receiving rifampin	0.008	−1.575	0.087	−0.893
CYP2B6 haplotypes				
*1/*1	0.001	−1.777	0.027	−1.084
*1/*2	0.220	−1.262		
*1/*4	0.638	−0.864		
*1/*6	0.133	0.811		
*2/*4	0.342	−2.126		
*2/*6	0.865	−0.381		
*4/*6	0.748	−0.512		
*5/*6	0.971	0.081		
*6/*6	<0.001	4.893	0.001	3.076
CYP2B6 nucleotide polymorphisms				
64C>T	0.105	−1.387		
499C>G				
516G▶T	<0.001	2.149		
785A>G	<0.001	1.947		
1375A>G				
1459C>T	0.500	−1.077		
3003C>T	0.754	0.183		
18492T>C	<0.001	−1.635		
21563C>T	<0.001	2.090		

[a] *CYP2B6* nucleotide polymorphisms were not included in the multivariate analysis model because of a strong correlation between nucleotide polymorphisms and haplotypes.

pharmacogenetic markers of *CYP2B6* and clinical factors on efavirenz concentrations as well as the effect of drug-drug interactions in patients coinfected with HIV and active tuberculosis who were receiving a antituberculosis regimen with or without rifampin.

Overall, the allelic frequencies of the *CYP2B6* SNPs detected in this study are consistent with a recent study in Thais (17). *CYP2B6*

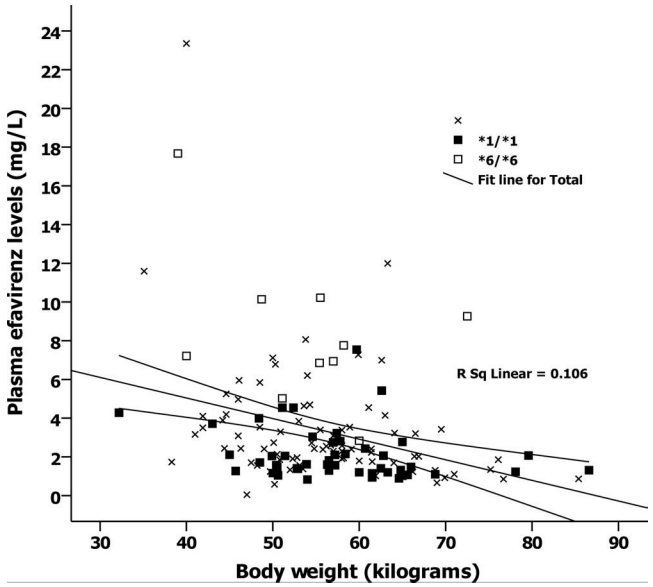

FIG 3 Relationship between body weight at the time of measurement and plasma efavirenz concentration by haplotype. Unfilled squares represent haplotype *6/*6, filled squares represent efavirenz haplotype *1/*1, and cross symbols represent other haplotypes. Broken lines represent regression predictions and 95% confidence intervals.

499C>G and 1375A>G were the absent SNPs in this study, and *CYP2B6* 1459C>T was rarely detected, which is consistent with previous reports from studies of Chinese patients (18, 19) and Japanese patients (16). No *CYP2B6* genetic polymorphism was found in one-third of all patients whose haplotype was determined to be *1/*1, and this haplotype was one of the risk factors associated with a low plasma efavirenz concentration by multivariate analysis. *CYP2B6* is genetically polymorphic; therefore, the determination of the haplotype would be another approach for predicting plasma efavirenz concentrations. Of note, the patients who carried *CYP2B6* *1/*2 and *2/*4 had a median plasma efavirenz concentration that was less than that seen with those who carried *CYP2B6* *1/*1, but those haplotypes were not found to be associated with low plasma efavirenz concentrations. Those data may be explained by the fact that only a small proportion of patients carried these genes. Taken together, these pharmacogenetic markers should raise concerns about maintaining an adequate drug concentration, especially in patients who have high body weight and concurrently receive rifampin. In addition, marked interpatient variability was observed during concurrent use of efavirenz and rifampin as well as during the use of efavirenz alone. This finding can be explained by the influences of these pharmacogenetic markers.

Another observation that arises from this study is that an increase in body weight after the time of initial measurement results in a decreasing efavirenz concentration. By multivariate analyses, efavirenz concentrations persistently decreased 0.7 mg/liter (coefficients = −0.07) for every 10-kg increase in patient body weight. A cross-sectional study from the Liverpool therapeutic drug monitoring registry and a study in Thais showed that body weight was an independent predictive factor for the plasma efavirenz concentration (20, 21), although some previous studies were not able to demonstrate this effect (22, 23). Moreover, patients' body weights increased over time while on treatment. Thus, a weight-based cutoff for efavirenz dosing is a reasonably practical therapeutic approach. To date, a body weight cutoff of 60 kg for the standard daily dosage of efavirenz has been proposed. According to the current U.S. Department of Health and Human Services (DHHS) guidelines, experts recommend a starting efavirenz dose of 600 mg/day and monitoring for virological response. The guidelines suggest considering an increase of the dose to 800 mg/day for patients who weigh more than 60 kg. On the other hand, rifampin is an essential drug for the treatment of tuberculosis and is also a potent inducer of expression of cytochrome P450 enzymes in the liver (8). Subtherapeutic efavirenz concentrations can occur when efavirenz is coadministered with rifampin, leading to subsequent treatment failure on an efavirenz-based antiretroviral regimen, as aforementioned (8). Approximately 7% of the patients who were and were not concurrently receiving rifampin had a plasma efavirenz concentration below the recommended concentration. Most patients in both groups achieved efavirenz concentrations above the minimum recommended concentration of 1 mg/liter. Thus, it is important that rifampin itself has a relatively small impact on the plasma efavirenz concentration compared to pharmacogenetic differences corresponding to *CYP2B6* and patients' body weights.

On the other hand, three SNPs, including 516G>T, 785A>G, and 21563C>T, appear to be associated with high plasma efavirenz concentrations, with all means of plasma efavirenz concentrations being greater than 7 mg/liter in the patients who carried homozygous mutants even if concurrently receiving rifampin. Heterozygous mutants of these SNPs showed the same effect but at a lesser magnitude. Although the *CYP2B6* 516G>T SNP is well known to be useful for predicting plasma efavirenz concentrations, little is known about the plasma efavirenz concentration prediction potential of the other two SNPs. Previous studies in Thais (13, 17) and members of other ethnic groups (12, 15, 24) showed high plasma efavirenz concentrations in HIV-infected patients with the *CYP2B6* 516TT genotype while receiving rifampin. The frequency of this allele ranges from 15% in Asians up to 50% in Africans (11, 15, 25).

A number of limitations of this study should be addressed. First, other genetic variations associated with the plasma efavirenz concentration, for instance, *CYP2B6* 983T>C, were not investigated in this study. An association with high efavirenz concentrations in Africans has been previously indicated for *CYP2B6* 983T>C (26); nonetheless, it was absent in Asians (18, 27). Second, other pathways of efavirenz metabolism via CYP2A6 in patients with impaired CYP2BC function have been previously reported (28). *CYP3A4* *1B was associated with lower efavirenz clearance. Neither *CYP3A4* nor *CYP2A6* SNPs were examined in this study; however, a relatively weak association between their variants and efavirenz concentrations was previously shown (13, 29). Third, data corresponding to the factor "receiving rifampin" did not reach a significant level, although this factor was found to be associated with low efavirenz concentrations in a previous study (30). This finding may have been due to a small sample size in the subgroup of patients who did not receive rifampin. Fourth, results from this study may not be completely applicable to members of other ethnic groups with inherited differences in their metabolisms. Ultimately, long-term treatment outcomes are needed to examine and confirm this finding.

This report provides interesting data regarding the factors potentially contributing to the pharmacokinetic variability of efavirenz in Thai patients coinfected with HIV and tuberculosis, including genetic factors, biological factors (i.e., body weight), and environmental factors (i.e., efavirenz-rifampin interactions). The patients with a particular haplotype and high body weight have the greatest probability of a low plasma efavirenz concentration. Pharmacokinetic variabilities reflect the combined influences and different magnitudes of such factors.

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We have no conflicts of interest to declare.

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Major Article

Title:

CYP2B6 g.18492T>C Polymorphism Compromises Efavirenz-based Antiretroviral Regimen in Co-infected HIV and Tuberculosis Patients Carrying *CYP2B6* Haplotype *1/*1

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Running title: *CYP2B6* and Low efavirenz level

Key words: *CYP2B6* g.18492T>C, efavirenz, HIV, tuberculosis, Thai

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Objective: To study *CYP2B6* g.18492T>C polymorphism that was associated with plasma efavirenz concentration and 48-week virologic response in HIV and tuberculosis (TB) co-infected patients.

Methods: Antiretroviral-naive HIV-infected Thai adults with active TB and all carried *CYP2B6* haplotype *1/*1 were prospectively enrolled to receive efavirenz 600 mg/tenofovir/lamivudine. A single nucleotide polymorphisms (SNP) *CYP2B6* g.T18492C were genotyped using real-time PCR. At 12 and 24 weeks after ART, plasma efavirenz concentrations at 12 hours after dosing were measured. Plasma HIV RNA was monitored every 12 weeks until 48 weeks.

Results: Of 139 patients, 48 patients carried haplotype *1/*1. Of 48 patients, mean±SD body weight was 56±10 kilograms and 77% received rifampicin-containing anti-TB regimen. No drug resistance-associated mutation was detected. The median (IQR) CD4 count was 41 (14–132) cells/mm^3 and median (IQR) plasma HIV RNA was 5.7 (5.3-6.1) log copies/mL. The frequencies of wild type, heterozygous, and homozygous mutant of *CYP2B6* g.18492T>C were 39%, 42%, and 19%, respectively. At 12 weeks, mean±SD efavirenz concentrations of patients who carried 18492TT, 18492TC, and 18492CC were 2.8±1.6, 1.7±0.9, and 1.4±0.5 mg/L, respectively (*P*=0.005). At 24 weeks, efavirenz concentrations of corresponding patients were 2.4±0.8, 1.7±0.8, and 1.2±0.4 mg/L, respectively (*P*=0.003). By multivariate analysis, low efavirenz concentration was associated with g.18492T>C SNP (β=-0.937, *P*=0.004) and high body weight (β=-0.032, *P*=0.046). At 48 weeks, 20% patients had treatment failure, including 12% virologic failure, 4% dead, and 4% lost to follow-up.

Conclusions: *CYP2B6* g.18492T>C polymorphism compromises efavirenz-based antiretroviral regimen in co-infected HIV and tuberculosis patients who carry *CYP2B6* haplotype *1/*1.

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INTRODUCTION

Efavirenz has been recommended as the preferred option for a non-nucleoside reverse transcriptase inhibitor (NNRTI) combined with other two nucleoside reverse transcriptase inhibitors in optimized first-line antiretroviral regimens [1]**.** This drug is primarily metabolized by the hepatic cytochrome P450 2B6 (CYP2B6) enzyme into 8-hydroxyefavirenz and the remaining via accessory pathways, involving CYP2A6, CYP3A4/5, and UGT2B7 [2-4]. On the other hand, rifamycin is a crucial component in the treatment of tuberculosis. Rifampicin is a strong hepatic cytochrome P450 inducer, resulting in a marked reduction of exposure of several antiretroviral drugs, including efavirenz but in a lesser magnitude [5]. Previous studies demonstrated favorable outcomes in co-infected HIV and tuberculosis patients with efavirenz-based antiretroviral therapy (ART) who had concurrently received rifampicin [6-9]. With a consequence, many current HIV treatment guidelines recommended to use efavirenz-based ART in the patients who are receiving rifampicin [1, 10, 11].

Nonetheless, concerns persist regarding variation of plasma efavirenz concentration in such patients. Polymorphisms in *CYP2B6*, resulting from a nucleotide substitution at some positions, are associated with lower rate of efavirenz metabolism and leads to high efavirenz exposure. Many studies have reported a relationship between *CYP2B6* c.516G>T variant and increased efavirenz concentration in plasma and intracellular compartments, as well as a higher risk of neuropsychiatric adverse events [12]. Conversely, variable activity of cytochrome P450 may result in lower drug clearance, and leads to suboptimal efavirenz concentration [12]. Resistant HIV quasispecies can rapidly emerge during suboptimal antiretroviral drug concentration [13-15]. However, data regarding pharamcogenetic marker correlated with suboptimal antiretroviral drug concentration and clinical

treatment outcomes is very limited. To date, the range of recommneded plasma efavirenz concentration at 12 hours after dosing is proposed to be 1-4 mg/L [1, 15]. Therefore, this study aim to investigate *CYP2B6* g.18492T>C polymorphism that was associated with plasma efavirenz concentration as well as 48-week virologic responses in patients who co-infected with HIV and tuberculosis.

MATERIALS AND METHODS

The previous study was a prospective, open-label trial [16] involving 139 adult Thai patients who had co-infected with HIV and tuberculosis to study the frequency of *CYP2B6* single nucleotide polymorphisms (SNPs) and haplotypes at Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand. The institutional ethics committees of Bamrasnaradura Infectious Diseases Institute and the Thai Ministry of Public Health approved the study. All participating patients had provided written, informed consents. The period of enrolment was between October 2009 and May 2011. They were followed until 48 weeks after initiation of ART to examined (1) pharmacogenetic marker of *CYP2B6* and biological factors which were associated with low plasma efavirenze concentration at week 12 while they were receiving rifampicin-containing anti-TB regimen and at week 24 while anti-TB regimen were discontinued and (2) immunologic and virologic responses at 48 weeks after ART initiation. Adherence were measured by self report and questionaire.

Briefly, initial inclusion criteria included: (1) HIV-infected patients 18-60 years of age, (2) newly clinically diagnosed active tuberculosis, positive acid-fast staining or a positive culture for *Mycobacterium tuberculosis*, (3) treated with antituberculous regimen 4 -12 weeks prior to enrollment, and (4) naïve to ART. All

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patients were started on once daily antiretroviral regimen of efavirenz 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 tuberculosis treatment. The dosage of rifampicin was 450 mg/day if body weight ≤50 kilograms and 600 mg/day for body weight >50 kilograms. Patients who received other anti-tuberculosis regimens without rifampicin were those who initially could not tolerate rifampicin due to adverse effects or hypersensitivity. The patients had follow-up visits at 2 weeks, 6 weeks, 12 weeks, 24 weeks, 36 weeks, and 48 weeks after initiation of ART, when they were assessed clinically and/or blood samples were taken. At the end of follow-up, virologic failure was defined as either a rebound plasma HIV-1 RNA of >1000 copies/ml after having a previously undetectable value or lack of achievement of undetectable level at 48 weeks of ART. Genotypic resistance testing (TRUGENE HIV-1 Genotyping Assay, Visible Genetics Inc., Toronto, Canada) was performed at week 0 and after the patient was documented with virologic failure.

At week 0, DNA was isolated from the stored EDTA cell pellets using the QlAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genomic DNA was quantified by a UV spectrophotometer ND-1000 at 260 nm (NanoDrop Technologies, Wilmington, DE). A total of eight SNPs within *CYP2B6* were genotyped. Seven of eight, including c.64C>T, c.499C>G, c.516G>T, c.785A>G, c.1375A>G, c.1459C>T, and g.21563C>T, were included for *CYP2B6* haplotype determination. Haplotype determination was interpreted using The Human Cytochrome P450 (*CYP*) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2b6.htm). *CYP2B6* g.18492T>C SNP identified using HapMap (www.hapmap.org) data on Japanese and Han Chinese populations with an r^2 >0.8 were assessment [17]. All sample were performed at Laboratory for Pharmacogenomics and Personalized Medicine,

Ramathobidi Hospital Mahidol University. Fasting plasma efavirenz concentration at 12 hours after taking was measured using a validated high performance liquid chromatopraphy assay at 12 weeks after ART inititation while receiving anti-tuberculosis treatment and at 24 weeks while discontinuation of tuberculosis treatment. All patients were instructed to strictly adhere their medications at least 2 weeks prior to measure plasma efavierenz concentrations. This assay was developed at the Department of Clinical Pharmacology at the University Medical Centre Nijmegen, The Netherlands. CD4 cell count by flow cytometry and plasma HIV-1 RNA by real-time polymerase chain reaction were assessed at baseline and every 12 weeks thereafter until 48 weeks.

Frequencies (%) and median (interquartile range at 25^{th} and 75^{th}, IQR) were used to describe clinical parameters and laboratory parameters. All possible risk factors associated with low plasma efavirenz concentration were evaluated with a linear regression model by adjusting for confounding factors. Any factors with P value <0.05 were included in the multivariate regression model. *P* value <0.05 was considered statistically significant. The beta value and its 95% confidence interval (CI) were estimated. Inter-patient variability of plasma efavirenz concentration was expressed as a coefficient of variation (CV). Genotype distributions were tested for Hardy-Weinberg equilibrium using exact tests. All analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Of 139 patients who had co-infected with HIV and tuberculosis, *CYP2B6* haplotype *1/*1 had been identified in 48 patients. Table 1 shows clinical characteristics, laboratory parameters, and pharmacogentic parameters of 48 patients.

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Mean±SD body weight was 56±10 kilograms and 77% had received rifampicin-containing anti-TB regimen. None of the patients had resistance-associated mutations to the study antiretroviral drugs prior to ART inititation. The frequencies of wild type, heterozygous, and homozygous mutant of *CYP2B6* g.18492T>C were 39%, 42%, and 19%, respectively. Figure 1 displays the scatter plots of plasma efavirenz concentrations by g.18492T>C SNP. At 12 weeks, inter-patient variabilities of plasma efavirenz concentrations at 12 weeks of the corresponding patients who carried 18492TT, 18492TC, and 18492CC were 57%, 53%, and 36%, respectively. Mean±SD plasma efavirenz concentrations of the corresponding patients were 2.8±1.6, 1.7±0.9, and 1.4±0.5 mg/L, respectively (P=0.005). The proportion of patients who had efavirenz concentraitons <1 mg/L were 0%, 15%, and 11%, respectively. At 24 weeks, inter-patient variabilities of plasma efavirenz concentrations at 12 weeks of patients who carried 18492TT, 18492TC, and 18492CC were 33%, 47%, and 33%, respectively. Plama efavirenz concentrations of patients who carried 18492TT, 18492TC, and 18492CC were 2.4±0.8, 1.7±0.8, and 1.2±0.4 mg/L, respectively (P=0.003). The proportion of patients who had efavirenz concentraitons <1 mg/L were 0%, 15%, and 22%, respectively. Mean±SD body weights at 12 and 24 weeks were 58±10, and 59±10 kilograms (P <0.001, compared to week 0), respectively.

Univariate analysis and multivariate analysis of possible factors associated with low plasma efavirenz concentrations at 12 weeks and 24 weeks are shown in table 2 and table 3, respectively. Overall, low plasma efavirenz concentrations at both 12 weeks and 24 weeks were associated with g.18492T>C SNP and high body weight (P<0.05). At 48 weeks, 20% of all patients had treatment failure, including 12% virologic failure, 4% dead, and 4% lost to follow-up. Among xx patients with drug

resistance, x (xx%) had mutations contributed to only NRTI resistance; x (x%) had mutations contributed to only NNRTI resistance; and x (xx%) patients had mutations contributed to both NRTI and NNRTI resistance. For NNRTI-resistance mutations, there were K103N (x of xx, x%) and Y181C/I (x of xx, xx%) There were no correlation between *CYP2B6* 18492T>C and virologic failure (*P*=0.841).

DISCUSSION

Many genetic polymorphisms associated with high efavirenz concentration have been identified in the *CYP2B6* among patients with HIV mono-infection [12]. This is the first study revealing that co-infected HIV and tuberculosis patients with *CYP2B6* haplotype *1/*1 who carried heterozygous and homozygous mutant of *CYP2B6* g.18492T>C had markedly low plasma efavirenz concentrations. Steady-state plasma efavirenz concentrations were reduced approximately 60% to 80% in the patients who had this allelic variant. Of note, means of efavirenz concentrations of the patients who carried heterozygous and homozygous mutants were 1.7 mg/L and 1.2 mg/L, respectively. Comparing to the previous study in patients with HIV mono-infection, efavirenz concentrations of the corresponding patients were 2.0 mg/L and 1.7 mg/L, respectively [17]. Patients in the present study were therefore more vulnerable. This finding can be explained by the effect of concurrent use of rifampicin. Another consideration is that although a number of patients remained achieve concentrations above the minimum recommended level at 1 mg/L but they were very marginal. These findings are consistent at both time points of efavirenz measurements. The consideration of lower bound concentration at 1 mg/L is based upon an acceptable 70% treatment response rate [15]. Whereas Stahle and colleagues suggested raising the lower bound to achieve at least 80% response rate [18]. Previous

studies have shown that patients who had experienced low or sub-therapeutic efavirenz concentrations increased the chance for the development of HIV resistant strains and leaded to subsequent treatment failure [15, 19, 20]. Moreover, efavirenz has a low genetic barrier to resistance; in fact, a single mutation results in high-level resistance not to only efavirenz but to other drugs in the same class as well [21]. Therefore, maintaining an adequate drug concentration is very important for achieving long-term virologic suppression and prevents collateral damage to other drugs. The frequencies of heterozygous and homozygous mutant of *CYP2B6* g.18492T>C accounted for 61% of this cohort. This propotion is considered to be relatively high. As aforementioned, rifampicin is an essential drug for treatment of tuberculosis and is a potent inducer of cytochrome P450 enzymes in the liver, resulting in reduced plasma concentrations of efavirenz [5]. Taken together, such patients are highly vulnerable to subsequent failure of HIV treatment, although no correlation was found between *CYP2B6* g.18492T>C and virologic failure after 48 weeks of treatment. A relatively small sample size in the present study is an explanation. Therefore, a larger scale of the study remains need to confirm.

In the present study, less inter-patient variability of efavirenz concentration was observed during concurrent use of efavirenz and rifampicin, and the consistency of this trend was found after rifampicin discontinuation. Substantial inter-patient variability of efavirenz concentration with >100% coefficient of variation after fixed standard dose is well known. The previous reports in Thais demonstrated high and wide ranges of inter-patient variability, ranging from 77-136% [6, 22]. It can be explained by that other SNPs had been excluded before the final analysis and only the patients who carried *CYP2B6* haplotype *1/*1 were enrolled, suggesting that other

potential factors that influence efavirenz concentration is minimized even if variation is usually even higher while co-administration with rifampicin [23, 24].

In addition, a high patients' body weight was found to be an important predictive factor of a low drug concentration. Plasma efavirenz concentrations persistently decreased at 0.9 mg/L for every 10 kilograms increase in body weight. The optimal efavirenz dosage when coadministered with rifampicin is still debated todate. According to the current US Department of Health and Human Services (DHHS) guidelines [1], experts recommend starting efavirenz dose of 600 mg/day and monitoring for virological response, and for patients weighting >60 kilograms, they would consider increasing the dose to 800 mg/day. The British HIV Association (BHIVA) treatment guidelines recommend using 50 kilograms as the cutoff for increasing the efavirenz dosage [11]. In contrast, World Health Organization guidelines for the resource-limited settings recommend using efavirenz at 600 mg/day only [10]. With a consequence, pharmacogenetic marker may play more roles to determine the appropriate dosage of efavirenz in the future, particularly in the settings of concurrent receiving rifampicin.

A number of limitations to this study should be considered. Firstly, the frequency of *CYP2B6* g.18492T>C in other ethnic is somewhat different based upon the HapMap (http://hapmap.ncbi.nlm.nih.gov/). Caucasians have more frequently found.

The genetic differences in the metabolism of efavirenz play roles on drug exposure. Thus, our results may not be completely applicable to ethnic group with different frequencies of polymorphisms. Secondly, small sample size is one of the limitations. It results in some factors reported in previous literatures cannot be indentified, such as concurrent receiving rifampicin [8]. Thirdly, pharmacogentic study is not performed

in other accessory pathways, including CYP2A6, CYP3A4/5, and UGT2B7 [25]. However, this study demonstrates that the pharmacogenetic marker and biological factors can influence the HIV treatment.

In conclusion, *CYP2B6* T to C substitution at gene position 18492 compromises efavirenz-based antiretroviral regimen in high body-weight co-infected HIV and tuberculosis patients who carry *CYP2B6* haplotype *1/*1. Further study with regard to integrating pharmacogenetic marker in the clinical practice should be considered.

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Table 1. Clinical characteristics, laboratory parameters, and pharmacogentic parameters of 48 co-infected HIV and tuberculosis patients

Characteristics	**Number** **n = 48**
Demographics	
Male gender	37 (77%)
Age, years, mean±SD	38 ± 8
Body weight, Kgs, mean±SD	56 ± 10
Receiving rifampicin-containing anti-tuberculous regimen	37 (77%)
Laboratory parameters	
CD4 cell count, cells/mm^3, median (IQR)	41 (14-132)
Percentage of CD4 cell count, %, median (IQR)	6 (2-12)
Log plasma HIV-1 RNA, Log copies/mL, median (IQR)	5.7 (5.3-6.1)
Hemoglobin, g/dL, median (IQR)	10.6 (9.2-12.2)
Serum alkaline phosphatase, mg/dL, median (IQR)	110 (71-191)
Aspartate aminotransferase, U/L, median (IQR)	35 (26-46)
Alanine aminotransferase, U/L, median (IQR)	24 (18-41)
Albumin, mg/dL, median (IQR)	3.4 (2.9-3.9)
Hepatitis B virus antigen: positive	3 (6%)
Hepatitis C antibody: positive	4 (8%)
Pharmacogenetic parameters	
T18492C SNP	
TT	19 (40%)
TC	20 (41%)
CC	9 (19%)

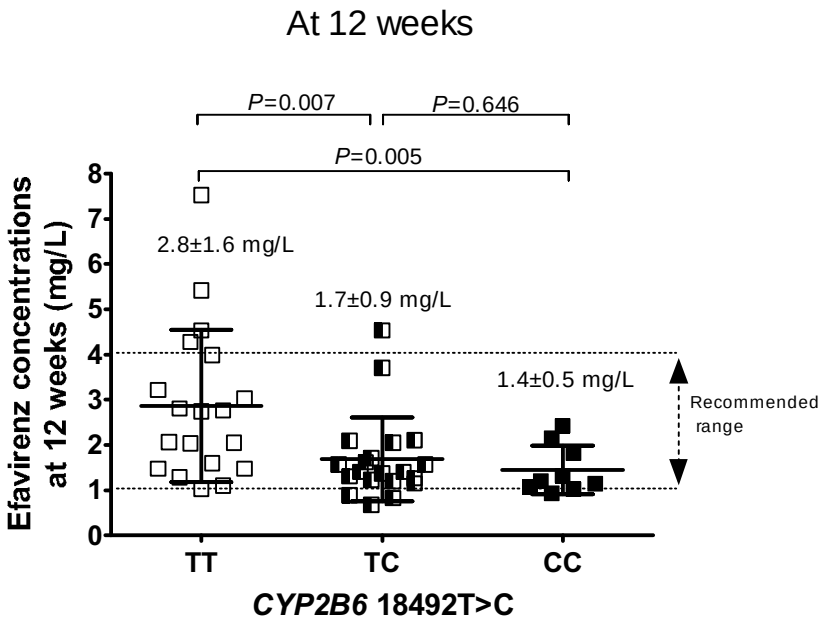
Table 2. Univariate and multivariate analysis of plasma efavirenz concentration at week 12 as the dependent variable

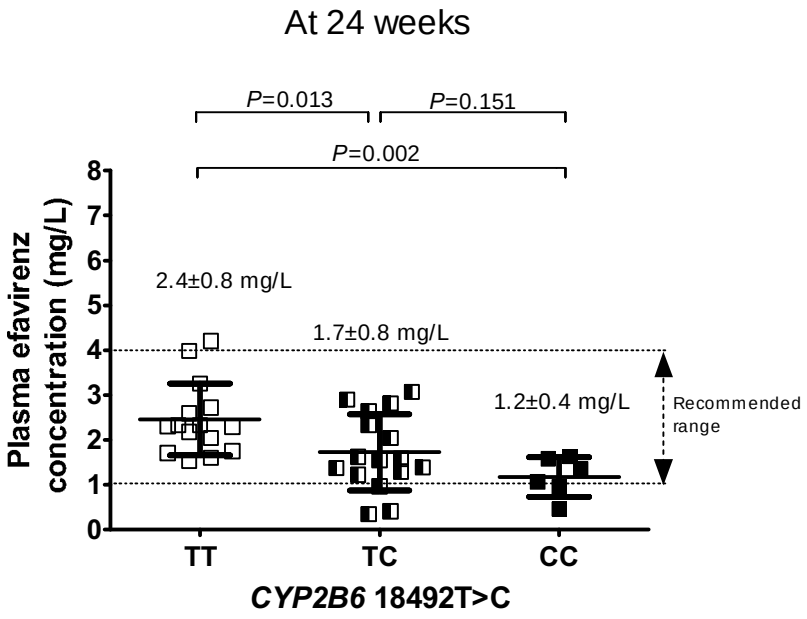
Parameters	Univariate analysis			Multivariate analysis		
	***P* value**	**Beta**	**95% CI**	***P* value**	**Beta**	**95% CI**
Demographics						
Male gender	0.407	0.395	-0.555 to 1.344			
Age	0.463	-0.018	-0.067 to 0.031			
Body weight at week 12	0.025	-0.039	-0.074 to -0.005	0.004	-0.948	-1.573 to -0.323
Extrapulmonary/disseminated TB						
Receiving rifampicin-containing regimen	0.321	-0.471	-1.418 to 0.475			
Laboratory parameters						
CD4 cell at week 0	0.215	0.003	-0.002 to 0.008			
Log plasma HIV RNA at week 0	0.734	0.116	-0.590 to 0.821			
Serum ALP at week 12	0.622	-0.001	-0.003 to 0.002			
AST at week 12	0.497	0.006	-0.011 to 0.022			
ALT at week 12	0.452	-0.007	-0.026 to 0.012			
Hepatitis B virus antigen: positive	0.042	1.651	0.064 to 3.238	0.508	-0.478	-1.924 to 0.967
Hepatitis C antibody: positive	0.919	0.074	-1.380 to 1.529			
Pharmacogenetic parameters						
T18492C SNP	0.001	-1.253	-1.986 to -0.519	0.004	-0.948	-1.573 to -0.323

Table 3. Univariate and multivariate analysis of plasma efavirenz concentration at week 24 as the dependent variable

Parameters	**Univariate analysis**			**Multivariate analysis**		
	***P* value**	**Beta**	**95% CI**	***P* value**	**Beta**	**95% CI**
Demographics						
Male gender	0.273	-0.368	-1.039 to 0.303			
Age	0.693	-0.008	-0.046 to 0.031			
Body weight at week 24	0.498	-0.010	-0.041 to 0.020			
Extrapulmonary/disseminated TB						
Laboratory parameters						
CD4 cell at week 0	0.754	-0.001	-0.005 to 0.003			
Log plasma HIV RNA at week 0	0.497	-0.174	-0.687 to 0.340			
Serum ALP at week 24	0.692	-0.001	-0.007 to 0.005			
AST at week 24	0.772	-0.001	-0.010 to 0.007			
ALT at week 24						
Hepatitis B virus antigen: positive	0.667	0.386	-1.480 to 2.252			
Hepatitis C antibody: positive	0.024	1.057	0.150 to 1.964	0.078	-0.762	-0..089 to 1.614
Pharmacogenetic parameters						
T18492C SNP	0.002	-0.881	-1.420 to -0.343	0.007	-0.764	-1.302 to -0.225

Figure 1. Scatter plots of plasma efavirenz concentrations at 12 weeks and 24 weeks by 18492T>C SNP. Each dot represents one patient. The middle bars indicates the means; and the upper and lower bars represent the standard deviations of means.





Major Article

Title:

CYP2B6 Haplotype and Biological Factors Responsible for Hepatotoxicity in HIV-infected Patients Receiving Efavirenz-based Antiretroviral Therapy

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Running title: *CYP2B6* and hepatotoxicity

Key words: HIV, hepatitis, *CYP2B6*, efavirenz, antiretroviral therapy

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ABSTRACT

Data on the pharmacogenetic marker of *CYP2B6* and biological factors which were associated with hepatotoxicity in HIV-infected patients receiving efavirenz-based antiretroviral regimen is 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 hours after dosing and liver chemistries were measured. Of all, mean±SD age was 37±8 years and 78% were male. The median (IQR) CD4 count was 43 (17–105) cells/mm^3. Eighteen (13%) patients had positive anti-HCV and 5 (4%) had positive HBsAg. The frequencies of wild type and heterozygous/homozygous mutant of each SNP were 64C>T (89%, 11%), 499C>G (100%, 0%), 516G>T (45%, 55%), 785A>G (37%, 63%), 1375A>G (100%, 0%), 1459C>T (96%, 4%), and 21563C>T (38%, 62%). Three most frequent haplotypes identified included *1/*6 (40%), *1/*1 (34%), and *6/*6 (8%). Median (IQR) plasma efavirenz concentration was 2.3 (1.4-3.7) mg/L. At 24 weeks, median (IQR) serum ALP was 98 (73-133) mg/dL, direct bilirubin was 0.11 (0.10-0.19) mg/dL. The proportion of grade 1 and grade 2 elevated serum ALP were 12.7% and 1.5%, respectively. By multivariate analysis, factors associated with high ALP, total bilirubin, and direct bilirubin at week 24 included *CYP2B6* haplotype *6/*6, high serum ALP at week 0, and positive anti-HCV (all P<0.05). In summary, HIV-infected Thai patients who have pharmacogenetic marker"*CYP2B6* haplotype *6/*6" may increase susceptibility to hepatotoxicity with efavirenz-based ART.

INTRODUCTION

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that is widely prescribed as part of combined antiretroviral therapy (ART) for the treatment of a human immunodeficiency virus (HIV) type 1, with the increased use in resource-constrained countries (1). Although effective ART currently increased life expectancy of HIV infected-patients, efavirenz-based ART has been associated with both short- and long-term toxicities, including hepatotoxicity (2, 3). However, frequencies of hepatotoxicity vary, depending on different antiretroviral regimens, immune status of patients, and comorbid conditions (2). Antiretroviral drug-associated hepatotoxicity manifests as either hepatocellular injury or hepatic cholestasis. A previous review has demonstrated that the frequency of grade 3 or 4 hepatotoxicity of efavirenz ranged from 1-8% (3-6). The current HIV treatment guidelines recommend monitoring of liver function every 3-6 months in HIV-infected patients who have been receiving ART (1)

Efavirenz is metabolized via hepatic cytochrome P450 (CYP), and it is both a substrate and inducer of the CYP2B6 and CYP3A4 isoforms of the CYP system (7). Variable activity of CYP results in inter-patient variability in drug clearance, efficacy as well as toxicity (7). To date, the range of recommneded plasma concentration of efavirenz at 12 hours after taking is proposed to be 1-4 mg/L (1, 8). With regard to pharmacogenetic factors and efavirenz-associated toxicity, several studies have shown the relationships among *CYP2B6* polymorphisms, high plasma efavirenz concentration, and neuropsychiatric adverse events (11). Data regarding *CYP2B6* haplotypes incorporated with plasma efavirenz concentration and biological factors related to efavirenz-associated hepatotoxicity is very scanty so far. In addition, frequency of *CYP2B6* mutant alleles and the incidence of hepatotoxicity varies among ethnics. This study therefore was conducted to examine

pharmacogenetic marker of *CYP2B6,* plasma efavirenz concentration, and biological factors which were associated with hepatotoxicity in Thai HIV- infected patients.

MATERIALS AND METHODS

The original study was a prospective, open-label trial involving 139 adult Thai patients co-infected with HIV and tuberculosis to study the frequency of *CYP2B6* single nucleotide polymorphisms (SNPs) and haplotypes at Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand (9). The institutional ethics committees of Bamrasnaradura Infectious Diseases Institute and the Thai Ministry of Public Health approved the study. The period of enrolment was between October 2009 and May 2011. All patients provided written, informed consent prior to enrollment. They were followed until 24 weeks after initiation of ART to examined pharmacogenetic marker of *CYP2B6,* plasma efavirenz concentration, and biological factors which were associated with hepatotoxicity.

The initial inclusion criteria included: (1) HIV-infected patients 18-60 years of age, (2) newly clinically diagnosed active tuberculosis, positive acid-fast staining or a positive culture for *Mycobacterium tuberculosis*, (3) treated with antituberculous regimen 4 -12 weeks prior to enrollment, (4) naïve to ART, (5) baseline CD4 cell count <350 cells/mm^3, and (6) participated and provided an informed consent. Exclusion criteria were as follow: (1) could not tolerate efavirenz due to any reason, (2) having serum alkaline phosphatase (ALP) >5 times of upper limit of normal range (ULN), aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) >5 times of ULN, (3) having serum creatinine >2 times of ULN (4) receiving immunosuppressive drugs, (6) being pregnant or lactation, and (7) patients who had prematurely discontinued efavirenz before week 12.

All patients were started on once daily antiretroviral regimen of efavirenz 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 tuberculosis treatment. The dosage of rifampicin was 450 mg/day if body weight ≤50 kilograms and 600 mg/day for body weight >50 kilograms. The standard antituberculosis regimen included isoniazid, rifampicin, pyrazinamide, and ethambutol for the first two months followed by isoniazid and rifampicin for the subsequent four to seven months. Patients who received other anti-tuberculosis regimens without rifampicin were those who initially could not tolerate rifampicin due to adverse effects or hypersensitivity. The patients had follow-up visits at week 2, week 6, week 12, and week 24 after initiation of ART, when they were assessed clinically and/or blood samples were taken. Hepatotoxicity was defined based on the AIDS Clinical Trial Group (ACTG): grade 0 (<1.25 ULN), grade 1 (≥1.25 ULN to ≤2.5 ULN), grade2 (>2.5 ULN to ≤5 ULN), grade 3 (>5 ULN to ≤10 ULN) or grade 4 (>10 ULN).

At baseline, DNA was isolated from the stored EDTA cell pellets using the QlAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). Genomic DNA was quantified by a UV spectrophotometer ND-1000 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 efavirenz concentrations (10) and *CYP2B6* SNP 21563 C>T were identified using the International Haplotype Mapping Project (HapMap) (http://www.hapmap.org) on Japanese and Han Chinese. SNP 499C>G was associated with high plasma efavirenz concentrations in Japanese and the remaining three SNPs were reported in Chinese, i.e., 64C>T, 1375A>G, and 1459C>T (11). *CYP2B6* haplotype determination was interpreted using The Human Cytochrome P450 (*CYP*) Allele Nomenclature Database (http://www.cypalleles.ki.se/cyp2b6.htm). All SNPs were included for *CYP2B6* haplotype interpretation.

Fasting plasma efavirenz concentration at 12 hours after taking was measured using a validated high performance liquid chromatopraphy assay at 12 weeks after ART inititation while receiving anti-tuberculosis treatment. This assay was developed at the Department of Clinical Pharmacology at the University Medical Centre Nijmegen, The Netherlands. CD4 cell count by flow cytometry and plasma HIV-1 RNA by real-time polymerase chain reaction, and liver chemistry were assessed at week 0 and at week 24 after ART.

Frequencies (%) and median (interquartile range at 25th and 75th, IQR) were used to describe clinical parameters and laboratory parameters. All possible risk factors associated with hepatotoxicity were evaluated with a linear regression model by adjusting for confounding factors. *P* value <0.05 was consedered statistically significant. The independent parameters those were strongly correlated between each other were not analyzed in the same multivariate analysis model. All analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

One hundred and thirty-four patients were enrolled and had follow-up until 24 weeks of ART. Patients' clinical parameters and baseline laboratory parameters of 134 patients are shown in Table 1. Serum ALP was 101 (73-168) mg/dL, AST was 38 (27-52) U/L, ALT was 31 (18-46) U/L, albumin was 3.4 (3.1-3.8) mg/dL. Of 134 patients, 99 (74%) received rifampicin-containing anti-tuberculosis regimens. The frequencies of wild type and heterozygous/homozygous mutant of each SNP were 64C>T (89%, 11%), 499C>G (100%, 0%), 516G>T (45%, 55%), 785A>G (37%, 63%), 1375A>G (100%, 0%), 1459C>T (96%, 4%), and 21563C>T (38%, 62%). Three most frequent haplotypes identified included *1/*6 (40%), *1/*1 (34%), and *6/*6 (8%). Median (IQR) plasma efavirenz concentration was 2.3 (1.4-3.7) mg/L; 7.2 (5.0-10.1) mg/L in those with haplotype *6/*6; and 2.1 (1.4-3.4) mg/L in

those with non*6/*6 (P<0.001). Overall, interpatient variability of plasma efavirenz concentration was 96%. At 24 weeks, median (IQR) serum ALP was 98 (73-133) mg/dL, AST was 31 (26-46) U/L, ALT was 30 (23-46) U/L, total bilirubin was 0.33 (0.25-0.47) mg/dL, direct bilirubin was 0.11 (0.10-0.19) mg/dL. Figure 1 displays the proportions of patients with abnormal liver chemistries. None of the patient had grade 4 abnormality.

Univariate and multivariate analyses of possible factors associated with high serum ALP level, total bilirubin, and direct bilirubin are shown in Table 2, Table 3, and Table 4, respectively. By multivariate analysis, predictive factors associated with high serum ALP included haplotype *6/*6, high baseline serum ALP, and positive anti-HCV antibody (P<0.05). The predictive factors associated with high total 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 direct bilirubin as a dependent variable (P<0.05). There were no relationship between high transaminase enzymes, including AST and ALT, and all study predictive parameters (P>0.05).

DISCUSSION

To date, the utility of phamacogentic marker to predict chance of efavirenz-associated hepatotoxicity is very limited. *CYP2B6* is genetically polymorphic (7,12-14), thus, the determination of haplotype could be a better approach than a SNP. This is the first study, showing a strong corellation between a predicitive pharmagenetic factor and hepatic cholestasis in HIV-infected patients who had been received efavirenz-based ART. Although no patient had symptomatic hepatitis but 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 of efavirenz toxicity

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(7). In addition, efavirenz is mainly metabolized via CYP2B6, and the lesser extent via other pathways, CYP3A4 and CYP2A6 for instances (15-17). Of note, hepatic cholestasis was a predominant abnormal hepatic profile found in this study. Rifampicin co-administration may play roles, owing to rifampicin itself can cause cholostasis, particularly in HIV-infected patients (18). Nevertheless, the parameter "receiving rifampicin" was not found to be significantly associated with hepatotoxicity because almost all of study patients had received rifampicin-containing anti-tuberculosis regimen. However, antiretroviral drug-associated hepatotoxicity manifests as either hepatocellular injury or hepatic cholestasis. On the other hand, markers of hepatocellular injury was not found to be associated with *CYP2B6* haplotype *6/*6 in the present study. A previous study have shown a correlation between this haplotype and transaminitis in Africans (19). Most of patients in this cohort had mild hepatotoxicity, either cholestasis or transaminitis without clinical symptoms. This finding may be explaind by that the present study enrolled patients who had relatively normal liver chemistry without advanced stage of liver disease and they all had been clinically stable with efavirenz-based ART for a period of time. However, this finding should be raised a clinical concern in the treatment of patients with chronic liver disease who receive efavirenz-based ART. Further studies are needed to confirm clinical utility in such patients.

One of the purposed mechanisms of non-nucleoside reverse transcriptase inhibitor-associated hepatotoxicity is dose-dependent mechanism (2). The observed high inter-patient variability of plasma efavirenz concentration may play role regarding hepatotoxicity. However, there appears to be no correlation between high plasma efavirenz concentration and hepatotoxicity by the multivariate analysis. A previous study revealed an association between high efavirenz concentration and transaminitis (19). The different frequencies of *CYP2B6* mutant allele between ethnics, relatively small sample size, and other unknown mechanism of efavirenz-induced hepatotoxicity may be the explanations of this unrelated

intermediate marker. Thus, only the factor "high efavirenz concentration" might not be able to explain hepatotoxic involvement.

Another observation is that hepatitis C virus (HCV) co-infection is a significant factor to predict hepatotoxicity. This finding is similar to other antiretroviral regimens in patients who had co-infected HIV and HCV, including protease inhibitor-based ART (20). HCV co-infection was an important determining factor, increasing 2- to 7-fold risk of hepatotoxicity (2). Severity of liver injury before and after initiating ART play roles. The potential of hepatotoxicity may be exacerbated by this co-infection, especially in the patients who carry *CYP2B6* haplotype *6/*6. On the other hand, hepatits B virus (HBV) co-infection was not found to be associated with hepatotoxicity. This finding can be explained by that tenofovir and lamivudine were a component of backbone nucleoside reverse transcriptase inhibitors used in the present study. They have antiviral activities against both HIV and HBV (21, 22). In addition, proportion of patients who co-infected with HBV was relatively small (4%) when compared to those co-infected with HCV (13%). Given that sex-dependent hepatotoxicity is correlated to nevirapine (2), female sex was not found to be associated with hepatotoxicity in the present study.

A number of limitations need to be addressed. First, this study excluded the patients who had early discontinued efavirenz due to adverse events, hepatotoxicity, for example. A previous study demonstrated that *CYP2B6* 516TT involved the early discontinuation of efavirenz also (23). Second, the mechanism of efavirenz-induced hepatotoxicity remains unclear except hypersensitivity reaction during the first few weeks of initiation. Further study remains need. Third, gamma-glutamyltranspeptidase level was not performed. However, elevations of other satisfy markers of hepatic cholestasis were indicative of hepatic cholestasis. Ultimately, other possible causes of cholestatic disease were not completely excluded, including infiltrative liver disease, biliary tract disease, and concurrent

opportunistic infections. Nevertheless, none of the patients subsequently developed other clinical significance.

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

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Table 1. Clinical characteristics and laboratory parameters of 134 co-infected HIV and tuberculosis patients

Characteristics	**Number** **n = 134**
Demographics	
Male gender	104 (78%)
Age, years, mean±SD	37 ± 8
Body weight, Kgs, mean±SD	54 ± 11

Sites of TB	
Lung	63 (47%)
Cervical lymph node	12 (9%)
Disseminated TB	54 (40%)
Meninges	3 (2%)
Colon	2 (1%)
Laboratory parameters	
CD4 cell count, cells/mm^3, median (IQR)	43 (17-105)
Percentage of CD4 cell count, %, median (IQR)	6 (3-11)
Log plasma HIV-1 RNA, Log copies/mL, median (IQR)	5.8 (5.4-6.3)
Hemoglobin, g/dL, median (IQR)	10.9 (9.7-12.0)
Serum alkaline phosphatase, mg/dL, median (IQR)	101 (73-168)
Aspartate aminotransferase, U/L, median (IQR)	38 (27-52)
Alanine aminotransferase, U/L, median (IQR)	31 (18-46)
Total bilirubin, mg/dL, median (IQR)	0.41 (0.30-0.71)
Direct bilirubin, mg/dL, median (IQR)	0.2 (0.1-0.4)
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)
Hepatitis B virus antigen: positive	5 (4%)
Hepatitis C antibody: positive	18 (13%)
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%

Table 2. Univariate and multivariate analysis of high serum ALP level at week 24 as the dependent variable

Parameters	**Univariate analysis**		**Multivariate analysis Model I***		**Multivariate analysis Model II**	
	***P* value**	**Beta**	***P* value**	**Beta**	***P* value**	**Beta**
Body weight at week 0	0.150	-0.865	-	-	-	-
Male gender	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	-	-	-	-
Hepatitis B virus antigen: positive	0.537	-20.771	-	-	-	-
Hepatitis C antibody: positive	0.001	61.024	0.026	34.408	0.019	36.748
Percentage of CD4 cell	0.042	1.845	0.255	0.837	0.359	0.689
Log plasma HIV RNA	0.256	11.499	-	-	-	-
Total cholesterol at week 0	0.956	0.009	-	-	-	-
Efavirenz concentration	0.088	3.475	-	-	0.129	2.582
CYP 2B6 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	-	-

*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.

Table 3. Univariate and multivariate analysis of high total bilirubin level at week 24 as the dependent variable

Parameters	**Univariate analysis**		**Multivariate analysis Model I***		**Multivariate analysis Model II**	
	***P* value**	**Beta**	***P* value**	**Beta**	***P* value**	**Beta**
Body weight at week 0	0.310	0.004	-	-	-	-
Male gender	0.054	0.181	0.156	0.119	0.094	0.148

Age	0.793	-0.001	-	-	-	-
Extrapulmonary/disseminated TB	0.598	0.042	-	-	-	-
Receiving rifampicin-containing regimen	0.461	0.064	-	-	-	-
Serum ALP at week 0	0.004	0.001	0.028	0.001	0.011	0.001
AST at week 0	0.454	-0.001	-	-	-	-
ALT at week 0	0.765	<0.001	-	-	-	-
Total bilirubin at week 0	0.495	0.066	-	-	-	-
Direct bilirubin at week 0	0.412	0.084	-	-	-	-
Hepatitis B virus antigen: positive	0.997	-0.001	-	-	-	-
Hepatitis C antibody: positive	0.001	0.393	0.017	0.253	0.007	0.302
Percentage of CD4 cell	0.711	0.002	-	-	-	-
Log plasma HIV RNA	0.924	-0.006	-	-	-	-
Total cholesterol at week 0	0.143	0.001	-	-	-	-
Efavirenz concentration	0.081	0.022	-	-	0.180	0.016
CYP 2B6 haplotypes						
*1/*1	0.401	-0.070	-	-	-	-
*1/*2	0.864	-0.026	-	-	-	-
*1/*4	0.540	-0.163	-	-	-	-
*1/*6	0.283	-0.086	-	-	-	-
*2/*4	0.547	-0.196	-	-	-	-
*2/*6	0.536	-0.201	-	-	-	-
*4/*6	0.600	-0.121	-	-	-	-
*5/*6	0.846	0.063	-	-	-	-
*6/*6	<0.001	0.665	<0.001	0.149	-	-

* Efavirenz concentration was not included in the multivariate analysis model I because of a strong correlation between efavirenz concentration and haplotype *6/*6.

Table 4. Univariate and multivariate analysis of high direct bilirubin level at week 24 as the dependent variable

Parameters	**Univariate analysis**		**Multivariate analysis Model I***		**Multivariate analysis Model II**	
	***P* value**	**Beta**	***P* value**	**Beta**	***P* value**	**Beta**
Body weight at week 0	0.550	0.052	-	-	-	-

Male gender	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	-	-	-	-
Hepatitis B virus antigen: positive	0.861	-0.015	-	-	-	-
Hepatitis C antibody: positive	<0.001	0.307	0.007	0.218	0.003	0.250
Percentage of CD4 cell	0.619	0.043	-	-	-	-
Log plasma HIV RNA	0.702	0.033	-	-	-	-
Total cholesterol at week 0	0.339	0.083	-	-	-	-
Efavirenz concentration	0.094	0.145	-	-	0.222	0.100
CYP 2B6 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	-	-

Efavirenz concentration was not included in the multivariate analysis model I because of a strong correlation between efavirenz concentration and haplotype *6/*6.

Figure 1. Proportion of patients with abnormal liver chemistries