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CYP3A Genotypes in Bangladeshi Tuberculosis Patients

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Abstract

The purpose of this study is to investigate the genotype and allelic frequencies of CYP3A in Bangladeshi Tuberculosis (TB) patients which may help for individualized drug dosing and improved therapeutics. Genotyping was done using the extracted genomic DNA from 90 TB patients followed by amplification of target alleles by Polymerase Chain Reaction (PCR). Amplified alleles were then digested by restriction enzymes followed by gel electrophoresis & sequencing to identify the targeted alleles namely *CYP3A4*1B, CYP3A4*2, CYP3A4*4, CY3A4*5, CYP3A4*6, CYP3A4*10, CYP3A4*18*, and *CYP3A5*3*. In TB patients, no samples were positive for *CYP3A4*2, CYP3A4*4, CYP3A4*5, CYP3A4*6, CYP3A4*10,* and *CYP3A4*18* alleles. One sample was found to be heterozygous for *CYP3A4*1B* (1.11%). The wild homozygous (*CYP3A5*1/*1*) genotype frequency was 7.78%, the heterozygous (*CYP3A5*1/*3*) frequency was 42.22% and the homozygous mutant (*CYP3A5*3/*3*) frequency was 50% in Bangladeshi TB patients. The absence of the common polymorphic gene suggests that there will be no impact of CYP3A drug metabolizing enzymes on antituberculosis drugs.

Introduction

Genetic polymorphisms in the drug metabolizing enzymes (DMEs) can result in varied interindividual and interethnic pharmacological and toxicological responses upon exposure to therapeutics and environmental pollutants[1]. Interindividual variation in drug metabolism is caused by many factors including environmental factors, concurrent drug therapy as well as genetic factors[2]. Much of this variation, however, has shown to be caused by genetic polymorphisms of the human cytochrome P450 enzymes (CYP)[3]. The cytochrome P450 (CYP) 3A subfamily plays a major role in the oxidative, peroxidative, and reductive biotransformation reactions of 50–60% of all currently used drugs[4,5], exogenous carcinogens, and endogenous substrates such as steroids[6,7]. The CYP3A subfamily composed of CYP3A4, CYP3A5, CYP3A7, and CYP3A43 in humans is of special importance because it accounts for as much as 30% of total liver cytochrome P450 content[8]. The most abundant CYP3A isoform in liver and intestine is CYP3A4. Its interindividual hepatic expression varies 60-fold[9], and the in vivo function as assessed by clearance displays at least a 20-fold difference[10]. Induction by xenobiotics (e.g. rifampin) and endogenous compounds (e.g. steroid hormones) further modulates the variability of CYP3A4 expression among individuals[11]. Although the substrate specificity of CYP3A5 is similar to that of CYP3A4, CYP3A5 has been regarded to be less important for drug elimination because it is expressed at much lower levels than CYP3A4[12]. This variability in CYP3A expression and function explains why the intensity and duration of drug action and the occurrence of side effects show large patient-to-patient variability[11].

To date, several polymorphic CYP3A isoforms have been described in different populations in particular the CYP3A4 and 3A5 isoforms[13-15]. The most common CYP3A4 variant reported so far, *CYP3A4*1B*, is an A-392G transition in the promoter region[16,17]. A number of rarer variants, mainly nonsynonymous polymorphisms, have also been reported[18]. CYP3A5 expression is polymorphic with a variant allele of CYP3A5, *CYP3A5*3*, conferring low or undetectable CYP3A5 expression as a result of a single point mutation (6986A>G)[12,16]. However, carriers of at least one wild type *CYP3A5*1* allele expresses CYP3A5 in the small intestine, liver and the kidneys[12,16,19].

Tuberculosis (TB) is a common and often deadly infectious disease usually caused by *Mycobacterium tuberculosis* in humans. Tuberculosis is a major public health problem in Bangladesh[20]. Different drugs, namely isoniazid,

rifampin, rifapentine, pyrazinamide, ethambutol (as first-line drugs), cycloserin, ethionamide, p-amino salicylic acid, streptomycin, capreomycin (as second-line drugs), are used for the treatment and management of tuberculosis[21]. Rifabutin (as first-line drugs) and levofloxacin, moxifloxacin, gatifloxacin, amikacin/kanamycin (as first-line drugs) are also used for the treatment of TB which are not approved by the United States Food and Drug Administration (FDA)[21].

Most antituberculosis drugs are liposoluble and their elimination requires biotransformation into more water-soluble compounds. This is mostly performed by hepatic phase I and phase II biotransformation enzymes. In the phase I reaction, oxidation or demethylation occurs, usually performed by cytochrome P450 (CYP450) enzymes. The compound is usually still not very water soluble, and requires further metabolism. Phase I reactions often produce toxic intermediates. In a typical phase II reaction, a large water-soluble compound is attached by glucuronidation or sulfation, resulting in non-toxic metabolites which can easily be eliminated[22]. To date, allelic frequencies and genotypes of CYP3A4 and CYP3A5 variant alleles have been reported in different ethnic groups[2,18,23-26]. In Bangladeshi healthy subjects, no sample (n=200) was positive for *CYP3A4*2, *4, *5, *6, *10* & **18* alleles[25]. Two samples heterozygous for *CYP3A4*1B* (1.0%) and twenty six samples with the genotype *CYP3A5*1/*1 (13.0%)* were found in Bangladeshi healthy subjects[25]. In North Indians, two heterozygotes with genotype *CYP3A4*1/1B* were found in the high enzyme activity group whereas *CYP3A4*2, *4, *5, *6* & **10* were absent (n=200)[18]. Hence, the present study has been designed to investigate the genotypes and allelic frequencies of CYP3A in Bangladeshi TB patients. SNPs, which are known to have genetic variation and can affect CYP3A activity among Asian subjects, are selected based on the findings of different published papers[2,18,23-26].

Materials and Methods

Subject selection

Ninety TB patients from Tuberculosis unit, National Institute of Diseases of Chest and Hospital (NIDCH), Dhaka, consisting of 61 men and 29 women were recruited for the study. Demographic data of all the TB patients are presented in Table I. The study was conducted between July 2009 to June 2010 in the Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka in accordance with the International Conference of Harmonization (ICH) for Good Clinical Practice (GCP) and in compliance with the Declaration of Helsinki and its further amendments[27,28]. Volunteers were informed about the experimental procedures and the study. Each volunteer signed an informed consent document before entering into the study and was free to withdraw from the study at any time without any obligation. Ethical permission was taken to approve the protocol and consent form of the clinical investigation from the Ethical Review Committee of National Institute of Diseases of Chest and Hospital (NIDCH), Dhaka.

Table I: Demographic data of TB patients (n=90)

	Mean	Standard Deviation	Range (min-max)
Age (years)	35.96	13.78	13-70
Body weight (Kg)	45.21	9.03	20-65
BMI (Kg/m^2)	17.39	3.85	8.6-27.6

Genomic DNA Isolation

About 3 ml of venous blood was drawn into a tube containing EDTA and stored at–20°C until the isolation of genomic DNA. Genomic DNA from all the blood samples was isolated[29]. The purity of the DNA and their concentrations were measured by UV-Spectrophotometer (Shimadzu, Tokyo, Japan) at 260 nm.

PCR-RFLP for CYP3A4 and CYP3A5 genotyping:

Primers required to genotype for *CYP3A4*1B, CYP3A4*2, CYP3A4*4, CY3A4*5, CYP3A4*6, CYP3A4*10, CYP3A4*18 and CYP3A5*3* were designed according to previously published papers[2,23-25]. A 25 µl PCR reaction volume containing 1 µl of genomic DNA (50-70 ng/µl), 5 µl of 5×GoTaq reaction buffer, 4 µl of $MgCl_2$ (25 mM), 2 µl of dNTPs (2.5 mM), 1 µl of each primer (10 µM), 0.1 µl of GoTaq DNA polymerase (5 U/µl) (Promega corporation, USA), and 10.9 µl of nuclease free water was used. After PCR amplification, 20 µl PCR products were digested (overnight at 37ºC) with approximately 2 units of *Mbo*II, *Xcm*I, *BsmA*I, *Cla*I, *Hinf*I, *HpyCH4*III, *Hpa*II & *Rsa*I for *CYP3A4*1B, *2, *4, *5, *6, *10, *18* and *CYP3A5*3*, respectively[2,23-25]. PCR primers, annealing temperatures, restriction enzymes used and length of the expected fragments on digestion to genotype different CYP3A4 & CYP3A5 alleles are presented in Table II. Electrophoresis was done for restriction enzyme digested products using polyacrylamide gels (10%) in 1×TBE buffer.

Table II: Primers, PCR conditions, restriction enzymes and expected DNA fragments on digestion to genotype different CYP3A4 and *CYP3A5*3* alleles (35 cycle PCR reactions)

Allele	Primers	Annealing Temperature	RE	DNA fragments
*CYP3A4*1B*	FP 5'-GGAATGAGGACAGCCATAGAGACAAGGGGA-3' RP 5'-CCTTTCAGCTCTGTGTTGCTCTTTGCTG-3'	57°C	*Mbo*II	AF 385 NH 175, 169, 41 HE 210, 175, 169, 41 MH 210, 175
*CYP3A4*2*	FP 5'-TGTTGCATGCATAGAGGAAGGATGG-3' RP 5'-ATGACAGGGTTTGTGACAGGG-3'	57°C	*Xcm*I	AF 450 NH 450 HE 450, 232, 218 MH 232, 218
*CYP3A4*4*	FP 5'-CACATTTTCTACAACCATGGAGACC-3' RP 5'-TTTATACCTGTCCCCACCAGATTC -3'	57°C	*BsmA*I	AF 249 NH 141, 94, 14 HE141, 94, 47, 14 MH 94, 47, 14
*CYP3A4*5*	FP 5'-TGTTGCATGCATAGAGGAAGGATGG-3' RP 5'-ATG ACA GGG TTT GTG ACA GGG -3'	57°C	*Cla*I	AF 450 NH 450 HE 450, 250,200 MH 250, 200
*CYP3A4*6*	FP 5'-GAGCCATATTCTCAGAAGGGAGATCAAG-3' RP 5'-CAAACATGTGTCGTTCTGCTATGTGG -3'	58°C	*Hinf*I	AF 290 NH 137, 129, 24 HE153, 137, 129, 24 MH 153,137
*CYP3A4*10*	FP 5'-ACTTACTGCTCCATGCTGGGGAAAG-3' RP 5'-TCTGGTCACTGGAATAACCCAACAGC-3'	60°C	*HpyCH 4*III	AF 280 NH 280, HE 280,178,102 MH 178, 102
*CYP3A4*18*	FP 5'-AATGATTTGCCTTATTCTGGTTCTG-3' RP 5'-TTTCACCTCCTCCCTCCTTCTC-3'	58°C	*Hpa*II	AF 388 NH 388 HE 388, 199, 189 MH 199, 189
*CYP3A5*3*	FP 5'-CCTGCCTTCAATTTTTCACT-3' RP 5'-GGTCCAAACAGGGAAGAGGT-3'	61°C	*Rsa*I	AF 196 NH 102, 74, 20 HE 102, 94, 74, 20 MH 102, 94

AF, Amplified fragment; NH, Normal homozygote; HE, heterozygote; MH, Mutant homozygote, RE, Restriction endonuclease

DNA sequencing

DNA sequencing was performed to further confirm the genotyping results. Six samples from each different genotype were chosen at random and sent for sequencing to the Centre for Advanced Research in Sciences, University of Dhaka, Bangladesh. Sequencing was also done for the sample containing *CYP3A4*1B* allele for further confirmation. The PCR products were purified using Biobasic PCR purification kit (Biobasic, Canada) before being sent for DNA sequencing by standard Kit of ABI PRISM BigDye® Terminator (Applied Biosystems, USA). The sequencing results were then verified against the published sequences for CYP3A4 and CYP3A5. GenBank Accession no. were AF185589 (*CYP3A4*1B*); AF209389 (*CYP3A4*2, *4, *5, *6, *10,* and **18*); J04813 (*CYP3A5*1*); AC005020 (*CYP3A5*3*).

Statistical analysis

The SPSS software package (Version 16.0, SPSS Inc., Chicago, Illinois, USA) was used to analyze the data. Descriptive statistics were used for all variables. Values were expressed as percentage, mean, and standard deviation. Data were compiled according to the genotype and allele frequencies[25].

Results

The purity (OD 260/OD 280) of all the genomic DNA was found to be in the range between 1.7 to 1.9 and the average concentration was 50 to 70 µgm/ml. In all reactions, correct lengths of expected PCR products were obtained. Restriction endonuclease *Mbo*II digestion fragments of *CYP3A4*1B* and *Rsa*I digestion fragments of *CYP3A5*3* in 10% Polyacrylamide gel were shown in Figure 1 and Figure 2, respectively.

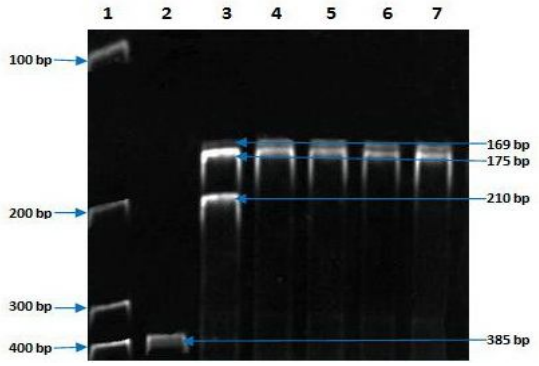

Fig. 1: Restriction Endonuclease (*Mbo*II) digestion fragment of *CYP3A4*1B* (Lane 3 to 7) (10% Polyacrylamide gel). Lane-1, Molecular ruler; Lane-2, uncut PCR product (385 bp); Lane-3, **1B* heterozygote (210, 175, 169, 41 bp); Lane-4 to 7, **1B* normal homozygote (175, 169, 41 bp).

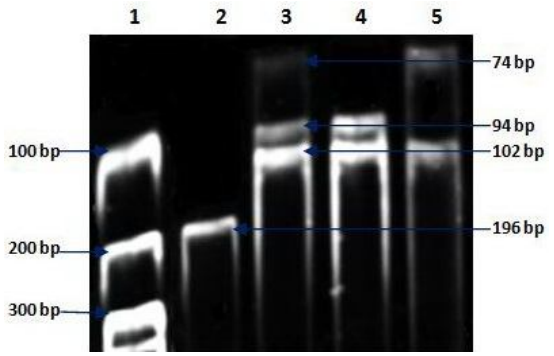

Fig. 2: Restriction Endonuclease (*Rsa*I) digestion fragment of *CYP3A5*3* (Lane 3 to 5) (10% Polyacrylamide gel). Lane-1, Molecular ruler; Lane-2, uncut PCR product (196 bp); Lane-3, **1/*3* (102, 94, 74, 20 bp); Lane-4, **3/*3* (102, 94 bp); Lane-5, *1/*1 (102, 74, 20 bp).

The genotype and allelic frequencies of CYP3A4 and CYP3A5 are presented in Table III. No samples were positive for *CYP3A4*2, CYP3A4*4, CYP3A4*5, CYP3A4*6, CYP3A4*10 and CYP3A4*18* alleles. One sample was heterozygous for *CYP3A4*1B* (overall allelic frequency was 0.56%) (n=90). The homozygous wild-type CYP3A5 (*CYP3A5*1/*1*) genotype frequency was 7.78% (7/90), the heterozygous (*CYP3A5*1/*3*) frequency was 42.22% (38/90) and the homozygous mutant (*CYP3A5*3/*3*) frequency was 50% (45/90) (Table III).

Table III: Frequencies of CYP3A4 and CYP3A5 variant alleles in the Bangladeshi TB patients (n=90)

Allele	Genotype frequency (%)			Allelic frequency (%)	
	W/W	W/M	M/M	W	M
*CYP3A4*1B*	98.89	1.11	0	99.44	0.56
*CYP3A5*3*	7.78	42.22	50	28.89	71.11

W=Wild, M=Mutant

Discussion

CYP3A is involved in the metabolism of more than 60% of all drugs used in human[30]. It has been well known that interindividual difference in metabolic profile of many drugs is mainly due to sequence variants in genes encoding different drug metabolizing enzymes as inherited determinants generally remain stable throughout a person's lifetime. Africans and Caucasians demonstrates *CYP3A4*1B* frequency of 60% and 4%, respectively, but has not been found in Chinese and Japanese[31,32]. A low allelic frequency of 0.56% was observed in the Bangladeshi Tuberculosis patient which is consistent with the allelic frequency of 0.56% found in 200 healthy Bangladeshi subjects[25]. DNA expressed *CYP3A4*2* demonstrated nine-fold decreased intrinsic clearance (V_{max}/K_m) of nifedipine as compared to *CYP3A4*1*, whereas $K_m$and V_{max} of *CYP3A4*2* were not significantly different from *CYP3A4*1* for testosterone hydroxylation[32]. *CYP3A4*2* occurs with a frequency of 2.7% in Caucasians and is absent in Africans and Chinese[32] and also in our study. Hsieh *et al.* [18] reported that, those with *CYP3A4*4*, *CYP3A4*5* and *CYP3A4*6* alleles have decreased CYP3A4 activity compared to those with no mutations shown. In 200 healthy Bangladeshi subjects, no allele was found to be positive for *CYP3A4*2*, *CYP3A4*4*, *CYP3A4*5*, *CYP3A4*6*, *CYP3A4*10*, *CYP3A4*18*[25]. Our studies in TB patients have also demonstrated the absence of these alleles so it can be assumed that there will be no effect on metabolism by CYP3A4. CYP3A5 gene is highly polymorphic in the Chinese, Malay, Indian and Bangladeshi populations with the **3* allele having a frequency of 76% in the Chinese population and about 60% in Malays and Indians and 50% in Bangladeshis[10,33]. Our study results are similar to that for Indians, Malays and Bangladeshi healthy subjects. The common polymorphic genes are absent in Bangladeshi population suggesting no impact of CYP3A drug metabolizing enzymes on antituberculosis drugs.

References

1. Dandara C, Masimirembwa CM, Magimba A, Sayi J, Kaaya S, Sommers De K, Snyman JR, Hasler JA. Genetic polymorphism of CYP2D6 and CYP2C19 in East and Sounthern African populations including psychiatric patients. Eur J Clin Pharmacol. 2001; 57: 11-17.

2. Ruzilawati AB, Mohd Suhaimi AW, Gan SH. Genetic polymorphisms of CYP3A4: *CYP3A4*18* allele is found in five healthy Malayan subjects. Clin Chim Acta. 2007; 383:158–162.

3. Tateishi T, Watanabe M, Moriya H, Yamaguchi S, Sato T, Kobayashi S. No ethnic difference between Caucasian and Japanese hepatic samples in the expression frequency of CYP3A5 and CYP3A7 proteins. Biochem Pharmacol. 1999; 57:935–939.

4. Guengerich FP. Cytochrome P-450 3A4: regulation and role in drug metabolism. Annu Rev Pharmacol Toxicol. 1999; 39:1–17.

5. Gibson GG, Plant NJ, Swales KE, Ayrton A, El Sankary W. Receptordependent transcriptional activation of cytochrome P4503A genes: induction mechanisms, species differences and interindividual variation in man. Xenobiotica. 2002; 32:165–206.

6. Waxman DJ. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. Arch Biochem Biophys. 1999; 369:11–23.

7. Yamazaki H, Shimada T. Progesterone and testosterone hydroxylation by cytochromes P450 2C19, 2C9, and

3A4 in human liver microsomes. Arch Biochem Biophys. 1997; 346: 161–169.

8. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther. 1994; 270: 414–423.

9. Ozdemir V, Kalow W, Tang BK, Paterson AD, Walker SE, Endrenyi L, Kashuba AD. Evaluation of the genetic component of variability in CYP3A4 activity: a repeated drug administration method. Pharmacogenetics. 2000; 10: 373–388.

10. Wilkinson GR. Cytochrome P4503A (CYP3A) metabolism: prediction of in vivo activity in humans. J Pharmacokinet Biopharm. 1996; 24(5): 475–490.

11. Burk O, Tegude H, Koch I, Hustert E, Wolbold R, Glaeser H, Klein K, Fromm MF, et al. Molecular mechanisms of polymorphic CYP3A7 expression in adult human liver and intestine. The Journal of Biological Chemistry. 2002; 277(27): 24280–24288.

12. Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, Nuessler AC, Neuhaus P, Klattig J, Eiselt R, Koch I, Zibat A, Brockmöller J, Halpert JR, Zanger UM, Wojnowski L. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics. 2001; 11: 773–779.

13. Hirth J, Watkins PB, Strawderman M, Schott A, Bruno R, Baker LH. The effect of an individual's cytochrome CYP3A4 activity on docetaxel clearance. Clin Cancer Res. 2000; 6:1255–1258.

14. Schuetz J, Beach P, Guzelian PS. Selective expression of cytochrome P450 CYP3A mRNAs in embyonic and adult human liver. Pharmacogenetics. 1994; 5:11–20.

15. Eiselt R, Domanski TL, Zibat A, Mueller R, Presecan-Siedel E, Hustert E, Zanger UM, Brockmoller J, Klenk HP, Meyer UA, Khan KK, He YA, Halpert JR, Wojnowski L. Identification and functional characterization of eight CYP3A4 protein variants. Pharmacogenetics. 2001; 11:447–458.

16. Rebbeck TR, Jaffe JM, Walker AH, Wein AJ, Malkowicz SB. Modification of clinical presentation of prostate tumors by a novel genetic variant in CYP3A4. J Natl Cancer Inst. 1999; 90:1225–1229.

17. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet. 2001; 27:383–391.

18. Hsieh KP, Lin YY, Cheng CL, Lai ML, Lin MS, Siest JP, Huang JD. Novel mutations of CYP3A4 in Chinese. Drug Metab Dispos. 2000; 29:268–273.

19. Dai Y, Iwanaga K, Lin YS, Hebert MF, Davis CL, Huang W, Kharasch ED, Thummel KE. In vitro metabolism of cyclosporine A by human kidney CYP3A5. Biochem Pharmacol. 2004; 68(9):1889–1902

20. Banu S, Gordon SV, Palmer S, Islam R, Ahmed S, Alam KM, Cole ST, Brosch R. Genotypic Analysis of Mycobacterium tuberculosis in Bangladesh and Prevalence of the Beijing Strain. J Clin Microbiol. 2004; 42(4):1861.

21. American Thoracic Society and Centers for Disease Control and Prevention and Infectious Disease Society of America. Treatment of tuberculosis. Am J Respir Crit Care Med. 2003; 167 (4):603–662.

22. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. 1995; 333:1118–27.

23. Rais N, Chawla YK, Kohli KK. CYP3A4 genotypes and phenotypes in North Indians. Eur J Clin Pharmacol. 2006; 62:417–422.

24. Hu YF, He J, Chen GL, Wang D, Liu ZQ, Zhang C, Duan LF, Zhou HH. *CYP4A5*3* and *CYP3A4*18* single nucleotide polymorphisms in a Chinese population. Clin Chim Acta. 2005; 253:187–92.

25. Maruf AA, Ahmed MU, Yasmin H, Ullah MA, Azad MAK, Daly AK, Hasnat A. Genotypes and phenotypes of CYP3A in Bangladeshi population, Clin Chim Acta. 2011; 412: 531-536.

26. King BP, Leathart JBS, Mutch E, Williams FM, Daly AK. CYP3A5 phenotype-genotype correlations in a British population. Br J Clin Pharmacol. 2003; 55: 625–629.

27. World Medical Association Declaration of Helsinki, *Ethical Principles for Medical Research Involving Human Subjects*. Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the 52nd WMA General Assembly, Edinburgh, Scotland, October 7, 2000 (Accessed Nov 10, 2010, at http://www.wma.net/e/policy/b3.htm).

28. European Agency for the Evaluation of Medicinal Products, International Conference on Harmonization - World Health Organization, *Guideline for Good Clinical Practice*. ICH topic E6. Geneva, Switzerland: WHO; 2002 (Accessed Nov 10, 2010, at http://www.emea.europa.eu).

29. Daly AK, Monkman SC, Smart J, Steward A, Cholerton S. Analysis of cytochrome P450 polymorphisms. Methods in Molecular Biology. 1998; 107: 405–422.

30. Thummel KE, O'Shea D, Paine MF, Shen DD, Kunze KL, Perkins JD, Wilkinson GR. Oral first-pass elimination of midazolam involves both gastrointestinal and hepatic CYP3A-mediated metabolism. Clin Pharmacol Ther. 1999; 59:491–502.

31. Ball SE, Scatina J, Kao J, Ferron GM, Fruncillo R, Meyer P, et al. Population distribution and effects on drug metabolism of a genetic variant in the 5′ promoter region of CYP3A4. Clin Pharmacol Ther. 1999; 66:288–294.

32. Sata F, Sapone A, Elizondo G, Stocker P, Miller VP, Zheng W, Raunio H, Crespi CL, Gonzalez FJ. CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. Clin Pharmacol Ther. 2000; 67:48–56.

33. Balram C, Zhou Q, Cheung YB, Lee EJD. *CYP3A5*3* and **6* single nucleotide polymorphisms in three distinct Asian populations. Eur J Clin Pharmacol. 2003; 59: 123–126.