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RAPID COMMUNICATION

Genetic variability of CYP2B6 polymorphisms in four southern Chinese populations

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Abstract

AIM: To investigate the genotype and allelic frequencies of Cytochrome P450 2B6 polymorphisms in four southern Chinese populations.

METHODS: DNA was obtained from blood samples from Han Chinese from Hong Kong and three minority groups, the Wa, Bulang and Lahu from Yunnan in southern China. Genotyping was performed using real-time PCR and confirmed by direct sequencing.

RESULTS: A total of 507 subjects from southern China were studied. Results showed there is a high prevalence of 516G > T (34.5%) in ethnic Chinese compared to literature reports on other Asian populations and Caucasians. The frequency of the 516TT genotype is higher in the Han majority (23.1%) than in three other ethnic minority groups (i.e., 7.4%, 9.1% and 15.8%) in southern China.

CONCLUSION: This was the first study to document the spectrum of CYP2B6 allelic variants and genotypes in a southern Chinese population. The 516G > T allele is associated with a defective metabolism of efavirenz (EFV), which therefore may predispose to drug toxicity. Treatment regimens for human immunodeficiency virus (HIV) and heroin addiction may need to be optimized in different populations because of the marked variability of the key metabolizing enzyme.

Key words: Polymorphism; Allele; Genotype; Ethnicity; Discrimination PCR

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INTRODUCTION

Human hepatic cytochrome P450 is involved in the metabolism of a variety of drugs, many of which contribute to public health control of diseases. Cytochrome P450 2B6 is one of the poorly characterized CYP isoforms, but the list of known substrates is growing[1]. Efavirenz (EFV), a non-nucleoside reverse transcriptase inhibitor commonly used in HIV treatment, is one of the substrates. Previous *in vitro* studies have shown that CYP2B6 allele 516G > T can alter enzyme expression levels and/or activity[2]. Allele 516G > T is associated with greater plasma EFV exposure during the first 24 wk of antiretroviral therapy, and an increase in central nervous system side effects especially during the first week[3]. The mean plasma EFV concentrations of patients with CYP2B6 *6/*6 genotype (516TT genotype together with 785GG genotype) was significantly higher than those of patients with *6 heterozygous genotypes and non-*6 alleles[4]. CYP2B6 also metabolizes nevirapine (another key drug used in HIV treatment and the prevention of mother-to-child HIV transmission), methadone, nicotine and other anticancer drugs, antidepressant drugs, and antimalarial drugs[5]. Methadone is used as a maintenance treatment for opiate addiction and is a useful means of reducing HIV risk in injection drug users. With the rising problem of HIV in drug users globally, the optimal dose of methadone may vary with CYP2B6 polymorphisms.

Ethnicity is an important variable accounting for interindividual variability as a result of polymorphisms, as has been demonstrated in many populations. In this

Table 1 CYP2B6 genotypes in four study populations

A	Lahu (n = 216)	Wa (n = 202)	Bulang (n = 198)	Hong Kong (n = 398)
Allelic frequency				
allele	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
64C > T	6.5 (3.2-9.8)	6.4 (3.1-9.8)	11.6 (7.2-16.1)	11.6 (8.4-14.7)
516G > T	31.5 (25.3-37.7)	40.1 (33.3-46.9)	29.3 (23.0-35.6)	35.9 (31.2-40.6)
B	Lahu (n = 108)	Wa (n = 101)	Bulang (n = 99)	Hong Kong (n = 199)
Genotype frequency				
Genotype	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
64 CC	89.8 (84.1-95.5)	91.1 (85.5-96.6)	82.8 (75.4-90.3)	81.9 (76.6-87.3)
CT	7.4 (2.5-12.3)	5 (0.7-9.2)	11.1 (4.9-17.3)	13.1 (8.4-17.7)
TT	2.8 (0-5.9)	4 (0.2-7.8)	6.1 (1.4-10.8)	5 (2.0-8.1)
516 GG	44.4 (35.1-53.8)	35.6 (26.3-45.0)	50.5 (40.7-60.4)	51.3 (44.3-58.2)
GT	48.1 (38.7-57.6)	48.5 (38.8-58.3)	40.4 (30.7-50.1)	25.6 (19.6-31.7)
TT	7.4[b] (2.5-12.3)	15.8 (8.7-23.0)	9.1[d] (3.4-14.8)	23.1 (17.3-29.0)

[b]P < 0.01, Hong Kong Chinese *vs* Lahu, Chi-square test; [d]P < 0.01, Hong Kong Chinese *vs* Bulang, Chi-square test.

study, we investigated the genotypes and allelic frequencies of two common 2B6 variants in four southern Chinese populations, and made comparisons with results reported in the literature.

MATERIALS AND METHODS

Study population

We collected blood samples from 199 healthy Hong Kong Chinese donors *via* Hong Kong Red Cross Blood Transfusion Service from October 2005 to May 2006. Blood samples from people of three ethnic minority groups from Bulang (99), Wa (101) and Lahu (108) were collected from Yunnan Province by Kunming Medical College from May to September 2005. Approval was obtained from the Ethics Committee of both the Chinese University of Hong Kong and Kunming Medical College.

DNA isolation, primers, probes and discrimination PCR

Genomic DNA was isolated from blood samples using QIAGEN QIAamp DNA Mini Blood Kit (Hilden, Germany) according to the manufacture's instructions. Primers and TaqMan MGB probes used for discrimination real-time PCR in this study were described by Tsuchiya *et al*[4]. The sequences of primers and probes were: 64C > T, CCTCAACAGGACTCTTGCTACTC (forward), AGGCGGTCATGGGTGTTAG (reverse), VIC-TGGTTCAGCGCCACC-MGB (wt), FAM-CTGGTTCAGtGCCACC-MGB (mut); 516G > T, TCATGGACCCCACCTTCCT (forward), GACGATGGAGCAGATGATGTTG (reverse), VIC-TTCCAGTCCATTACC-MGB (wt), FAM-CTTCCAtTCCATTACC-MGB (mut). PCR reaction was carried out using an ABI 7500 Real Time PCR System with the following conditions: 50℃ for 2 min, 95℃ for 10 min, then 40 cycles of 95℃ for 15 s and 60℃ for 40 s. The SNPs were analyzed using ABI 7500 Real Time SDS software.

Sequencing

For direct sequencing, 2 sets of primers were designed for amplification of genomic DNA fragments and sequencing in this study. Set 1 primers (forward, 5'-A CCCACACACCCACACATTCACTTGCT-3'; reverse, 5'-TCCACACAGGCTGCA AACTGCTCAGAT-3') were used to amplify a genomic fragment containing exon 1 and a part of the promoter region. 64 C > T polymorphisms were explored from this set of sequences. Set 2 primers (forward, 5'-GCAGAGTGAGAACCGGCTGCATGGA-3'; reverse, 5'-TCCCTCCAGGTCTCCATGTCCCTG-3') were used to amplify a DNA fragment containing exon 4 and its flanking introns, and to explore 516G > T polymorphisms.

Genotyping

Genotyping was conducted by real-time PCR and confirmed by direct sequencing. The GeneBank accession numbers of CYP2B6 references used in this study are NG_000008 and NM_000767.

Statistical analysis

Fisher's exact test and chi-square test (χ^2) with Yates correction were used where appropriate, using the SPSS version 13.0 software package. $P < 0.05$ was considered to be statistically significant.

RESULTS

Demographics of the study groups

A total of 507 subjects from southern China were studied. Males accounted for 52%, 90%, 70%, and 47% of ethnic Lahu, Wa, Bulang, and Hong Kong Chinese, respectively. The median age was 25, 27, 32 and 30 years, respectively.

Allele frequency and genotype distribution

Two commonly reported alleles were evaluated in this study and the frequencies of 64C > T and 516G > T are listed in Table 1A. The frequency of the 64C > T allele was slightly higher in Bulang (11.6%) and Hong Kong (11.6%) compared to Lahu (6.5%) and Wa (6.4%). The overall 516G > T frequency was 34.5%, with a higher level in Wa (40.1%) than the other groups (Lahu 31.5%, Bulang 29.3%, and Hong Kong 35.9%). There was no significant difference in the frequency of the two alleles across the 4

Table 2 Comparison of allele 516G > T frequency between ethnic minority group and other ethnic populations

	Population	*n*	Frequency of allele 516G > T
Southern Chinese (current study)		1014	0.345
Caucasian	Germany[6]	242	0.252[a]
	Germany[6]	1146	0.26[a]
	American[7]	86	0.22[a]
	Switzerland[8]	418	0.24[a]
	Canadian[9]	354	0.254[a]
	European-American[3]	178	0.224[a]
	United Kingdom[10]	270	0.281[a]
	Germany[4]	430	0.286
African	African-American[7]	58	0.28
	African-American[11]	90	0.278
	African-American[3]	100	0.38
	African-Canadian[9]	246	0.329
	Ghanaians[11]	82	0.488[a]
Hispanics	Spanish[12]	200	0.265[a]
	Hispanic-American[7]	14	0.43
	Hispanic-American[3]	30	0.266
	Hispanic-Canadian[9]	122	0.328
Asian	Japanese[13]	530	0.16[a]
	Japanese[11]	90	0.144[a]
	Japanese[14]	176	0.2[a]
	Korean[11]	92	0.152[a]
	Taiwan[11]	92	0.141[a]
	Han Chinese[15]	386	0.21[a]
	Hong Kong[16]	152	0.43[a]

n: Total number of alleles. [a]$P < 0.05$, Southern Chinese *vs* other ethnic population, Chi-square test.

groups. The genotypic distributions of the alleles in the minorities are in Hardy-Weinberg equilibrium.

Table 1B shows the distribution of CYP2B6 genotypes in our study populations. The prevalence of the 516TT genotype in Hong Kong Chinese (23.1%) was significantly higher than in the Lahu and Bulang groups ($P < 0.01$).

Comparison of CYP2B6 allele 516G > T frequencies between the ethnic minority groups in Yunnan and the other ethnic groups

We compared the allelic frequency of 516G > T in our southern Chinese populations (34.5%) with other ethnic groups reported in the literature (Table 2). The frequency was significantly higher than for all other Asian populations, including Japanese, Korean, Taiwan, and Chinese-Han. The difference was also demonstrated with Caucasian populations including 2 series from Germany, Switzerland, the United Kingdom, and 3 Caucasian-American populations. No significant difference was found between results for our study populations and most Hispanic and African populations, except for a Spanish cohort and Ghanaians.

DISCUSSION

In this present study, we analyzed the genetic variability of the CYP2B6 gene in four ethnic Chinese groups: Han Chinese from Hong Kong and three ethnic minorities in Yunnan. There are 56 official ethnic groups in China, accounting for 8% of the total population. In Yunnan, a third of the population of 44 million belong to an ethnic minority. The three selected ethnic minority groups in this study are also related to populations in South East Asian countries, including Myanmar and Thailand. Our study revealed no significant difference in the frequencies of the two alleles 64C > T and 516G > T between the minority ethnic groups and the Han majority (Hong Kong Chinese). The 516TT genotype was, however, more prevalent in the Hong Kong group than in the Lahu and Bulang groups.

Our study is the first to document the distribution of CYP2B6 allelic variants and genotypes in a spectrum of southern Chinese populations, providing evidence that the 516G > T allele frequency is similar to Hispanics and African populations but markedly different from those of other Asian and Caucasian populations[3,4,6-14]. Our result differs from another study of Han Chinese, which reported a similar allelic frequency for 516G > T compared to other Asians, including Japanese and Korean, but was significantly different from American Caucasians[15]. A difference in sampling may account for the discrepancy between the studies. As a majority ethnic group, the Han Chinese recruited in Guangdong Province in south China might have come from other northern provinces. While the same may also be true for the Hong Kong Chinese in our study, blood donors are more likely to be young and truly local residents. On the other hand, the three minority ethnic groups comprise people living in isolated mountainous areas in Yunnan Province. Our results confirm that of a previous study yielding an even higher frequency for 516G > T 0.43, which might have arisen from a smaller sample size[16].

In our study, the 516TT genotype in Hong Kong Chinese is more prevalent than in Lahu and Bulang Chinese, suggesting that some ethnic groups might have higher plasma EFV concentrations during HIV treatment, a phenomenon already reported in African-Americans and Hispanics[3]. With the scaling up of HIV treatment around the world, variability in the pharmacokinetics of antiretroviral drugs may affect outcomes of treatment programmes. To better understand the phenotype-genotype association, pharmacokinetic analyses involving different ethnic Chinese people are needed. Our results on the pattern of CYP2B6 polymorphism in southern Chinese provided the rationale for follow-up research. It is, for example, important to determine the extent that different alleles of CYP2B6 among ethnic groups can affect enzymatic activity in the liver. Such research is even more important in places where there is a dual epidemic of HIV and heroin addiction. The widespread use of the latter in a harm reduction programme may create unique patterns of drug interactions that are associated with ethnic variation in CYP2B6 polymorphism. In some cases, individualization of drug therapy may be indicated when there is concurrent use of antiretroviral drugs, such as efavirenz and opiate agonists like methadone. Treatment optimization is becoming an important strategy in the development of HIV treatment guidelines in different countries, particularly multi-ethnic countries.

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