Allele and Genotype Frequencies of *Cytochrome P450 2B6* Gene in a Mongolian Population

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Nonstandard abbreviation:

CYP2B6: cytochrome P450 2B6
ABSTRACT

Cytochrome P450 2B6 (CYP2B6) plays an important role in metabolizing various drugs in common clinical use. Increasing interest in CYP2B6 genetic polymorphism was stimulated by revelations of a specific CYP2B6 genotype significantly affecting the metabolism of efavirenz, an anti-human immunodeficiency virus type-1 (HIV-1) agent. The present study determined the CYP2B6 haplotype in 100 healthy unrelated Mongolian volunteers by analyzing the genotypes of 9 single nucleotide polymorphism (SNP) positions (-82T>C, 64C>T, 499C>T, 516G>T, 777C>A, 785A>G, 983T>C, 1375A>G, and 1459C>T) in the CYP2B6 gene. The CYP2B6 *1 allele was the most frequent in the Mongolian population tested at 64.5%, higher than the equivalent frequency in African-Americans and Ghanaians. The second-most frequent allele was CYP2B6 *6 (21.0%), although this was less frequent than that in Ghanaians. Only one CYP2B6 *5 allele was identified in our Mongolian subjects (0.5%), although it is the third most frequent allele in Caucasian and African-American populations. These CYP2B6 genotypes revealed 7 slow efavirenz metabolizers in 100 Mongolians, which is significantly fewer than the same group among Ghanaians. Overall, the Mongolian CYP2B6 allele distribution was comparable with that in Japanese, Koreans, and Han Chinese. This is the first report of CYP2B6 genotype frequency in a Mongolian population, and could provide clinically useful information on drug metabolism in this population group.
INTRODUCTION

The cytochrome P450 2B6 (CYP2B6) gene encodes a member of the cytochrome P450 superfamily and has been mapped to the CYP2 gene cluster on chromosome 19 (Hoffman et al., 2001). The CYP proteins are mono-oxygenases that catalyze many reactions during the metabolism of numerous endogenous and exogenous compounds including drugs (Rendic, 2002; Porter, 2004). CYP2B6 was first identified in human liver microsomes and is expressed in certain extrahepatic tissues including kidney, intestine, lung, brain, trachea, and nasal mucosa (Mimura et al., 1993; Ding and Kaminsky, 2003; Miksys et al., 2003). Human CYP2B6 was originally thought to account for a minor portion (< 1%) of the total hepatic CYP content. However, subsequent studies showed the average relative contribution of CYP2B6 to total hepatic CYP content ranges from 2% to 10% (Wang and Tompkins, 2008).

CYP2B6 plays an important role in the metabolism of various commonly used drugs: the anti-cancer agents, cyclophosphamide and ifosfamide; the antiretrovirals, efavirenz and nevirapine; the anesthetics, propofol and ketamine; the antidepressant and antismoking agent bupropion; the antiestrogen tamoxifen; the synthetic opioid methadone; the anti-Parkinsonian selegiline; and others (Wang and Tompkins, 2008; Zanger and Hoffman, 2008). Interest in CYP2B6 research was recently stimulated by the ever-increasing list of substrates for this enzyme as well as polymorphic and ethnic variations in the expression and/or activity of the enzyme. Currently, 29 different alleles of the CYP2B6 gene are listed on the Human Cytochrome P450 Allele Nomenclature Committee website (http://www.cypalleles.ki.se/cyp2b6.htm).
Bupropion in vivo clearance via CYP2B6 *4 was reported to be higher compared to wild-type allele *1 (Kirchheiner et al., 2003), and slower efavirenz clearance was reported in CYP2B6 *6, *9, *16, *26, *27, and *28 carriers (Tsuchiya et al., 2004; Wang et al., 2006; Gatanaga et al., 2007; Rotger et al., 2007). Reduced CYP2B6 protein expression was found in human liver samples of *5 and *7 carriers (Lang et al., 2001). Lower expression was also found in cell lines transfected with *6, *11, *12, *13, *14, *15, *16, *18, *19, *20, and *21 alleles (Lang et al., 2004; Klein et al., 2005; Wang et al., 2006; Hofmann et al., 2008). Higher expression was detected *22-transfected cells (Zukunft et al., 2005). *27 allele was reported to result in decreased in vitro enzymatic activity of bupropion hydroxylase, and *28 was reported to result in truncated protein (Rotger et al., 2007).

The frequencies of different variant alleles of CYP2B6 have been studied in Caucasians, African-Americans, Japanese, Chinese, and Koreans, but no data are available for the Mongolian population. This study was designed to determine the genotypes and allelic frequencies of CYP2B6 in Mongolians, and to compare the results with those for other ethnic groups reported in the literature.
MATERIALS AND METHODS

Study population

We collected buffy-coat blood samples from 100 randomly selected, healthy, and unrelated Mongolian volunteers at the National Center for Communicable Diseases of Mongolia (NCCD). The ethics committees of the Ministry of Health, Mongolia, and the International Medical Center of Japan (IMCJ) approved the study protocol. All volunteers provided written informed consent.

CYP2B6 genotyping

Genomic DNA was isolated from the buffy coats using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) according to the instructions supplied by the manufacturer. For SNP analysis of the CYP2B6 coding region, the -82T>C, 64C>T, 499C>T, 516G>T, 777C>A, 785A>G, 983T>C, 1375A>G, and 1459C>T SNPs were genotyped using the allele-specific fluorogenic 5'-nuclease chain reaction assay with predesigned primers and TaqMan MGB probes (TaqMan SNP Genotyping Assay; Applied Biosystems, Foster City, CA) or previously published primers and MGB probes (Tsuchiya et al., 2004; Klein et al., 2005; Zukunft et al., 2005; Gatanaga et al., 2007) using the ABI PRISM 7900 HT Real-Time PCR System (Applied Biosystems). All PCR reactions were performed in a 25-µl volume containing 20 ng genomic DNA, 2 x TaqMan universal master mix (Applied Biosystems), and 20 x drug metabolism genotyping assay mix (Applied Biosystems) or 40 x assay mix (Applied Biosystems). PCR reactions were run as follows: 95°C for 10 min, then either 50 cycles of 92°C for
15 sec and 60°C for 90 sec (with drug metabolism genotyping assay mix, Applied Biosystems) or 40 cycles of 92°C for 15 sec and 60°C for 60 sec (with assay mix, Applied Biosystems).

**Statistical analysis**

Allele and haplotype designations were performed according to the published recommendation of the Human Cytochrome P450 Allele Nomenclature Committee (http://www.imm.ki.se/CYPalleles/criteria.htm). Data were compiled according to the genotype and allele frequencies estimated from the observed numbers of minor alleles at each polymorphic locus. All genotype distributions were confirmed to be in Hardy-Weinberg equilibrium. The frequency of \( CYP2B6 \) genotype in our subjects is presented with the 95% confidence interval (CI). Obtained data were compared with previously reported representative data in other ethnic groups. Differences in allele frequencies between ethnic groups were tested by Fisher’s exact test using the StatView software version 5.0 (SAS Institute, Cary, NC). A \( P \) value <0.01 was considered statistically significant.
RESULTS AND DISCUSSION

We successfully isolated genomic DNA from the 100 buffy coats and determined the genotypes of nine SNP positions (-82T>C, 64C>T, 499C>T, 516G>T, 777C>A, 785A>G, 983T>C, 1375A>G, and 1459C>T) in CYP2B6 (Table 1). No CYP2B6 genetic polymorphism was detected in 43 individuals and their haplotype was determined to be *1/*1. The haplotypes of single-SNP carriers with 64C>T, 785A>G, and 1459C>T were determined to be *1/*2, *1/*4, and *1/*5, respectively, while those of homozygous carriers with both 516TT and 785GG were determined to be *6/*6. Once it was established that *2, *3, *22, and *26 were the only alleles harboring 64C>T, 777C>A, -82T>C, and 499C>G, respectively, individuals with 64CT, 516GT, and 785AG; -82TC and 777CA; and 499CG, 516GT, and 785AG, were identified as *2/*6, *3/*22, and *1/*26 heterozygotes, respectively. Individuals carrying both 516GT and 785GG genotypes but not other polymorphisms, were determined to have *4/*6 heterozygotes. There were 22 Mongolians carrying 516GT and 785AG genotypes without other polymorphisms and there were two possible haplotypes, *1/*6 and *4/*9, in this genotypic pattern. That the 516GT genotype always coexisted with 785AG or 785GG and that the 516TT genotype always coexisted with 785GG indicated that the allele harboring 516T must have also carried 785G, and no *9 allele, which carries 516T and 785A, should have been present in this study group. Therefore, the 22 Mongolians with both 516GT and 785AG genotypes were *1/*6 heterozygotes. The 983T>C and 1375A>G polymorphisms,
which are the determinants of *18 and *23, respectively, were not observed in our subjects.

The most frequent CYP2B6 allele in the Mongolian population tested here was *1 (64.5%), similar to other ethnic groups including Japanese (Gatanaga et al., 2007), Koreans (Klein et al., 2005), Han Chinese (Guan et al., 2006), Caucasians (Jacob et al., 2004), and African-Americans (Klein et al., 2005). After statistical analysis, the frequency remained significantly higher than those in African-Americans and Ghanaians (Klein et al., 2005) (Table 2). The second most frequent allele in the Mongolian subjects was *6 (21.0%), although this was significantly less frequent than in Ghanaians, in whom the *6 allele is the most frequent. The third- and fourth-most frequent alleles were *4 and *2, respectively, as found in Japanese, Koreans, and Han Chinese, while *4 was not observed at all in African-Americans and Ghanaians. Only one CYP2B6 *5 allele was identified in our Mongolian subjects (0.5%), despite being the third-most frequent allele in Caucasians (12.2%) and African-Americans (8.6%).

Therefore, the present study revealed a comparable allele distribution for CYP2B6 in Mongolians to that in Japanese, Koreans, and Han Chinese, but one of significant difference from those in Caucasians, African-Americans, and Ghanaians.

Some CYP2B6 alleles have a major impact on the pharmacokinetics and pharmacodynamics of some drugs. It was reported that homozygotic carriers of CYP2B6 *6, *9, *16, *26, *27, and *28 are efavirenz slow metabolizers, and that dose reduction of efavirenz may improve central nervous system (CNS)-related symptoms such as dizziness and depression induced by high efavirenz concentration in patients with these genotypes (Tsuchiya et al., 2004; Wang et al., 2006; Gatanaga et al., 2007;
Rotger et al., 2007). The present subjects included seven efavirenz slow metabolizers deduced from *CYP2B6* gentyping, which is smaller than the same group in Ghanaians (Klein et al., 2005). This is the first report of *CYP2B6* genotype frequency in a Mongolian population, and the findings should contribute clinically useful information about drug metabolism and thus help to individualize drug treatment in Mongolians.

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References


Footnotes

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Table 1. CYP2B6 allele and genotype frequency in Mongolian population

<table>
<thead>
<tr>
<th>CYP2B6 Allele</th>
<th>Nucleotide position</th>
<th>Frequency (%</th>
<th>95% CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1 T C C G C A T A C</td>
<td>95% CI: 95% confidence intervals.</td>
<td>129 (64.5)</td>
<td>57.9 – 71.1</td>
</tr>
<tr>
<td>*2 T T C G C A T A C</td>
<td></td>
<td>7 (3.5)</td>
<td>1.6 – 7.2</td>
</tr>
<tr>
<td>*3 T C C G A A T A C</td>
<td></td>
<td>1 (0.5)</td>
<td>0 – 2.8</td>
</tr>
<tr>
<td>*4 T C C G C G T A C</td>
<td></td>
<td>18 (9.0)</td>
<td>5.7 – 14.2</td>
</tr>
<tr>
<td>*5 T C C G C A T A T</td>
<td></td>
<td>1 (0.5)</td>
<td>0 – 2.8</td>
</tr>
<tr>
<td>*6 T C C T C G T A C</td>
<td></td>
<td>42 (21.0)</td>
<td>15.4 – 26.6</td>
</tr>
<tr>
<td>*22 C C C G C A T A C</td>
<td></td>
<td>1 (0.5)</td>
<td>0 – 2.8</td>
</tr>
<tr>
<td>*26 T C G T C G T A C</td>
<td></td>
<td>1 (0.5)</td>
<td>0 – 2.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CYP2B6 Genotype</th>
<th>Nucleotide position</th>
<th>Frequency (%</th>
<th>95% CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1/*1 TT CC CC GG CC AA TT AA CC</td>
<td></td>
<td>43 (43.0)</td>
<td>33.1 – 53.3</td>
</tr>
<tr>
<td>*1/*2 TT CT CC GG CC AA TT AA CC</td>
<td></td>
<td>5 (5.0)</td>
<td>1.6 – 11.3</td>
</tr>
<tr>
<td>*1/*4 TT CC CC GG CC AG TT AA CC</td>
<td></td>
<td>14 (14.0)</td>
<td>7.9 – 22.4</td>
</tr>
<tr>
<td>*1/*5 TT CC CC GG CC AA TT AA CT</td>
<td></td>
<td>1 (1.0)</td>
<td>0.01 – 5.4</td>
</tr>
<tr>
<td>*1/*6 TT CC CC GT CC AG TT AA CC</td>
<td></td>
<td>22 (22.0)</td>
<td>14.3 – 31.4</td>
</tr>
<tr>
<td>*1/*26 TT CC CG GT CC AG TT AA CC</td>
<td></td>
<td>1 (1.0)</td>
<td>0.01 – 5.4</td>
</tr>
<tr>
<td>*2/*6 TT CT CC GT CC AG TT AA CC</td>
<td></td>
<td>2 (2.0)</td>
<td>0.2 – 7</td>
</tr>
<tr>
<td>*3/*22 TC CC CC GG CA AA TT AA CC</td>
<td></td>
<td>1 (1.0)</td>
<td>0.01 – 5.4</td>
</tr>
<tr>
<td>*4/*6 TT CC CC GT CC GG TT AA CC</td>
<td></td>
<td>4 (4.0)</td>
<td>1 – 9.9</td>
</tr>
<tr>
<td>*6/*6 TT CC CC TT CC GG TT AA CC</td>
<td></td>
<td>7 (7.0)</td>
<td>2.8 – 13.9</td>
</tr>
</tbody>
</table>

95% CI: 95% confidence intervals.
Table 2. CYP2B6 allele frequencies in ethnic populations.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Mongolians (n=200)</th>
<th>Japanese $^a$ (n=884)</th>
<th>Koreans $^b$ (n=88)</th>
<th>Han Chinese $^c$ (n=386)</th>
<th>Caucasians $^d$ (n=270)</th>
<th>African-Americans $^b$ (n=70)</th>
<th>Ghanaians $^b$ (n=64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>64.5% $^e$</td>
<td>67.4% $^e$</td>
<td>76.1% $^e$</td>
<td>67.1% $^e$</td>
<td>53.7%</td>
<td>44.3%</td>
<td>39.1%</td>
</tr>
<tr>
<td>*2</td>
<td>3.5</td>
<td>4.6</td>
<td>3.4</td>
<td>3.4</td>
<td>3.7</td>
<td>4.3</td>
<td>3.1</td>
</tr>
<tr>
<td>*3</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>*4</td>
<td>9.0</td>
<td>7.8</td>
<td>4.5</td>
<td>9.1</td>
<td>2.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>*5</td>
<td>0.5</td>
<td>0.8</td>
<td>0.0</td>
<td>0.3</td>
<td>12.2</td>
<td>8.6</td>
<td>1.6</td>
</tr>
<tr>
<td>*6</td>
<td>21.0$^f$</td>
<td>17.6$^f$</td>
<td>15.9$^f$</td>
<td>18.4$^f$</td>
<td>28.2</td>
<td>32.8</td>
<td>46.9</td>
</tr>
<tr>
<td>*22</td>
<td>0.5</td>
<td>ND</td>
<td>0.0</td>
<td>ND</td>
<td>ND</td>
<td>1.1</td>
<td>ND</td>
</tr>
<tr>
<td>*26</td>
<td>0.5</td>
<td>1.4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$-$^d$ representative data of the literature; $^a$ (Gatanaga et al., 2007); $^b$ (Klein et al., 2005); $^c$ (Guan et al., 2006); $^d$ (Jacob et al., 2004).

ND: not determined. $^e$ significantly higher than those in African-American and Ghanaians. $^f$ significantly less frequent than those in Ghanaians.