SNP Communication

Three Novel Single Nucleotide Polymorphisms (SNPs) of the CYP2B6 Gene in Japanese Individuals

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Summary: We sequenced all exons and exon-intron junctions of the CYP2B6 gene from 200 Japanese individuals. We found three novel single nucleotide polymorphisms (SNPs) (1375A→G, 1427G→A and 1454A→T) causing amino acid substitutions (Met→Val, Gly→Asp and Gln→Leu in exon 9), respectively.

The detected SNP was as follows:
1) SNP, 031226Hiratsuka01; GENE NAME, CYP2B6; ACCESSION NUMBER, AC023172; LENGTH, 25 base; 5′-CAGAACTTCTCCAGTGGCCAGCCCCG-3′.
2) SNP, 031226Hiratsuka02; GENE NAME, CYP2B6; ACCESSION NUMBER, AC023172; LENGTH, 25 base; 5′-CAGGGAGTGTGACGTGGGCAAAAT-3′.
3) SNP, 031226Hiratsuka03; GENE NAME, CYP2B6; ACCESSION NUMBER, AC023172; LENGTH, 25 base; 5′-CCCCAACATACCAGATCCGCTTC-3′.

Key words: CYP2B6; genetic polymorphism; Japanese

Introduction

CYP2B6 is involved in the metabolism of clinically important drugs; including cyclophosphamide,1 ifosfamide,2 bupropion,3,4 tamoxifen,5 and diazepam.6 The capacity of the activation has been reported to vary among individuals from four to nine times. Recent publications indicate that the extensive interindividual variability of CYP2B6 expression and function is due not only to regulatory phenomena, but also to a common genetic polymorphism.7 In addition to the wild-type allele CYP2B6*1, to date there are eight variants, designated CYP2B6*2 (64C→T), CYP2B6*3 (77C→A), CYP2B6*4 (785A→G), CYP2B6*5 (1459C→T), CYP2B6*6 (516G→T and 785A→G), CYP2B6*7 (516G→T, 785A→G and 1459C→T), CYP2B6*8 (415A→G), and CYP2B6*9 (516G→T) (see http://www.imm.ki.se/CYPalleles/).8 Recently, we have reported allele frequencies for CYP2B6*2–7 in 256 Japanese subjects using genotyping methods.9 In this study, we sequenced all exons and exon-intron junctions of the CYP2B6 gene from 200 Japanese individuals. We identified three novel SNPs of the CYP2B6 gene.

Materials and Methods

Venous blood was obtained from 200 unrelated Japanese volunteers of Tohoku University Hospital. The local Ethics Committee of Tohoku University Hospital and Tohoku Pharmaceutical University approved the study and written, informed consent was obtained from all blood donors. DNA was isolated from anticoagulated (with K2EDTA) peripheral blood using a DNA Extractor WB-Rapid Kit (Wako Pure Chemical Industries, Osaka, Japan) or a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s recommendations. Table 1 lists the primer pairs (PCR) used to amplify


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SNP1 (155)
CYP2B6 exons. These primers were designed based on the genomic sequence reported in the Genbank with accession number: AC023172. Amplicons for exons 1, 4, 7–8, and 9 were generated with the AmpliTaq Gold PCR Master Mix by Applied Biosystems (Foster City, CA, USA). The thermal profile consisted of denaturation at 94°C for 30 seconds, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 64.3°C for 90 seconds, and a final extension at 72°C for 7 minutes. The annealing temperatures are indicated in Table 1. Amplicons for exons 2–3 were generated with the Ex-Taq DNA polymerase by TaKaRa Co. (Kyoto, Japan). The thermal profile consisted of denaturation at 94°C for 30 seconds, annealing at 65°C for 60 seconds, extension at 72°C for 90 seconds, and a final extension at 72°C for 7 minutes. Amplicons for exons 5–6 were generated with the Ex-Taq DNA polymerase by TaKaRa Co. (Kyoto, Japan). The thermal profile consisted of denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 64.3°C for 60 seconds, extension at 72°C for 90 seconds, and a final extension at 72°C for 7 minutes.

Sequences were determined using the CEQ8000® automated DNA sequencer (Beckman-Coulter Inc., Fullerton, CA, USA). The PCR products were sequenced by the fluorescent dideoxy termination reaction method using the DTCS DNA Sequencing Kit (Beckman-Coulter Inc.) according to manufacturer’s instructions with the primers (sequence) listed in Table 1.

### Results and Discussion

We found three novel SNPs as follows:

1) SNP, 031226Hiratsuka01; GENE NAME, CYP2B6; ACCESSION NUMBER, AC023172; LENGTH, 25 base; 5'-CAGAACCTCTCC/GATGGC-CAGC CCCG-3'.
2) SNP, 031226Hiratsuka02; GENE NAME, CYP2B6; ACCESSION NUMBER, AC023172; LENGTH, 25 base; 5'-CAGAACCTCTCC/GATGGC-CAGC CCCG-3'.
3) SNP, 031226Hiratsuka03; GENE NAME, CYP2B6; ACCESSION NUMBER, AC023172; LENGTH, 25 base; 5'-CAGAACCTCTCC/GATGGC-CAGC CCCG-3'.

The electropherograms of the novel SNPs are shown in Table 1. The positions of these SNPs were 459 (031226Hiratsuka01), 476 (031226Hiratsuka02), and 485 (031226Hiratsuka03) in exon 9. All of the SNPs were heterozygous, and their frequencies were 0.0075 for 031226Hiratsuka01, 0.005 for 031226Hiratsuka02, and 0.0025 for 031226Hiratsuka03 in the Japanese population. Sequences from each sample were obtained from at least three different PCR amplifications.

Ariyoshi et al. have reported that the 516G>T (Gln172His) mutation enhances 7-ethoxycoumarin O-deethylase activity of the CYP2B6 enzyme in an in vitro assay.10 Further, Jinno et al. have also reported that compared with CYP2B6*, the alleles CYP2B6*4 (Lys262Arg), CYP2B6*5 (Arg262Cys), CYP2B6*6 (Gln262His; Lys262Arg), and CYP2B6*7 (Gln262His; Lys262Arg; Arg262Cys) are associated with a higher intrinsic clearance of 7-ethoxy-4-trifluoromethylcoumarin.11 Xie et al. have reported that the CYP2B6*6 carrier has significantly higher cyclophosphamide 4-hydroxylation.12 In contrast Lang et al. have reported that

### Table 1. Primers used for amplification and sequencing of the CYP2B6 gene

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer (5' to 3')</th>
<th>Reverse primer (5' to 3')</th>
<th>Annealing Temp. (°C)</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1</td>
<td>cccagtgcctctgaggctct</td>
<td>tttttcctctcccagacc</td>
<td>64.3</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>Exon 2</td>
<td>gctctcggtctgcccatctataaac</td>
<td>tttctgggtgttttcacctcacccccacacc</td>
<td>60.0</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>Exon 3</td>
<td>cccagtgcctctgaggctct</td>
<td>tttttcctctcccagacc</td>
<td>64.3</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>Exon 4</td>
<td>gctctcggtctgcccatctataaac</td>
<td>tttctgggtgttttcacctcacccccacacc</td>
<td>60.0</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>Exon 5</td>
<td>cccagtgcctctgaggctct</td>
<td>tttttcctctcccagacc</td>
<td>64.3</td>
<td>PCR and sequence</td>
</tr>
<tr>
<td>Exon 6</td>
<td>gctctcggtctgcccatctataaac</td>
<td>tttctgggtgttttcacctcacccccacacc</td>
<td>60.0</td>
<td>PCR and sequence</td>
</tr>
</tbody>
</table>

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**References**

1. Ariyoshi et al. have reported that the 516G>T (Gln172His) mutation enhances 7-ethoxycoumarin O-deethylase activity of the CYP2B6 enzyme in an in vitro assay.
2. Jinno et al. have also reported that...
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Fig. 1. The nucleotide sequences of the CYP2B6 gene at exon 9. The sequences are shown for sense strands. Arrows indicate the variant nucleotide positions.

1375 A>G  
(Met459Val)  
TTCTCCATGGCCA

1427 G>A  
(Gly476Asp)  
AGTGTGGTGGGG

1454 A>T  
(Gln485Leu)  
CATACCAGATCCG

1459 C>T  
(Arg487Cys)  
TTCTCCA>GTCGCCA

AGTGTGGTGGGG

CATACCAGATCCG

significantly reduced CYP2B6 protein expression and S-mephenytoin N-demethylase activity were found in carriers of the 1459C>T (Arg487Cys) mutation (alleles CYP2B6*5 and CYP2B6*7). Furthermore, it has been reported that the 1459C>T (Arg487Cys) are associated with the lowest level of CYP2B6 activity in livers of females. The 1459C>T SNP in exon 9 are closely located in the substrate recognition site. These three SNPs in this study are also located in the site. In particular, the Gly476 is conserved in the CYP2 family. Thus, these amino acid substitutions are expected to alter the catalytic properties of the CYP2B6.

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References


