SNP Communications

Two Novel Single Nucleotide Polymorphisms (SNPs) of the CYP2D6 Gene in Japanese Individuals

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: We analyzed all the exons and exon-intron junctions of the CYP2D6 gene from 286 Japanese individuals. We detected two novel single nucleotide polymorphisms (SNPs) 2556C>T (Thr261Ile) and 3835A>C in exon 8 (Lys404Gln). Both these SNPs showed a frequency of 0.002.

Key words: CYP2D6; genetic polymorphism; Japanese

Introduction

CYP2D6 metabolizes more than 50 clinically important drugs including some tricyclic antidepressants, neuroleptics, and \( \beta \)-adrenergic blockers. The CYP2D6 gene locus is extremely polymorphic, with more than 80 allelic variants (http://www.imm.ki.se/CYPalleles/cyp2d6.htm). The homozygous of defective CYP2D6 alleles, which result in the absence of CYP2D6 enzyme activity, are classified as poor metabolizer (PM) phenotypes. The frequency of PMs is 5%–10% in Caucasian population and less than 1% in Asian population. Among the variant alleles reported to date, three alleles, CYP2D6*3, CYP2D6*4, and CYP2D6*5, have been reported to account for approximately 95% of the alleles of PMs in Caucasian population. However, as yet, PMs associated with CYP2D6 function in the Japanese population could not be accounted for by the known variant alleles of CYP2D6.

In the present study, we analyzed all the exons and exon-intron junctions of the CYP2D6 gene from 286 Japanese individuals by using denaturing HPLC (DHPLC). Additionally, we identified two novel SNPs of the CYP2D6 gene in Japanese individuals.

Materials and Methods

Venous blood was obtained from 286 unrelated healthy Japanese volunteers and patients admitted to Tohoku University Hospital. Written informed consent was obtained from all the blood donors, and the study was approved by the Local Ethics Committee of Tohoku University Hospital and Tohoku Pharmaceutical University. DNA was isolated from anticoagulated (with K2EDTA) peripheral blood by using QIAamp DNA Mini Kits (Qiagen, Hilden, Germany) according with the manufacturer’s instructions.

Long PCR was performed in order to amplify the entire CYP2D6 gene and to detect the CYP2D6*5 allele using primer pairs (Table 1), as described by Johansson et al. Genomic DNA (10–50 ng) was amplified using LA-Taq DNA polymerase (TaKaRa Co., Kyoto, Japan). The amplification was performed on an iCycler (Bio-Rad, Hercules, CA, USA). The resultant PCR products were a 5-kb fragment contained the entire CYP2D6 gene and a 6-kb fragment that indicated the presence of the CYP2D6*5 allele. The thermal profile consisted of denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for...
Table 1. Primers used for the amplification of the entire CYP2D6 gene and identification of the CYP2D6*5 allele

<table>
<thead>
<tr>
<th>5' Primer</th>
<th>3' Primer</th>
<th>Annealing Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>entire CYP2D6 gene</td>
<td>ccagaaggtttcagagcctca</td>
<td>actgacccctggagagttgta</td>
</tr>
<tr>
<td>CYP2D6*5 allele</td>
<td>gccaccttcgctcagcctc</td>
<td>ggcagctcagcagcacc</td>
</tr>
</tbody>
</table>

Table 2. Amplification and DHPLC conditions for CYP2D6 SNP analysis of genomic DNA

<table>
<thead>
<tr>
<th>Exon</th>
<th>Size (bp)</th>
<th>5' Primer</th>
<th>3' Primer</th>
<th>Predicted Temp. (°C)</th>
<th>DHPLC Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>280</td>
<td>gtggggtgcagagcctgc</td>
<td>ggtgaggggcagcctgcgc</td>
<td>63.4</td>
<td>63.4, 66.0</td>
</tr>
<tr>
<td>2</td>
<td>272</td>
<td>agcggtgacgcttcagc</td>
<td>cacccacccagagtccccagc</td>
<td>64.7</td>
<td>64.7, 67.0</td>
</tr>
<tr>
<td>3</td>
<td>253</td>
<td>gtggggtgacgcgcggc</td>
<td>gttcggggccacgtccag</td>
<td>65.4</td>
<td>65.4</td>
</tr>
<tr>
<td>4</td>
<td>261</td>
<td>aggggagggcagagcgc</td>
<td>acctcgggctcggcagcag</td>
<td>65.4</td>
<td>65.4</td>
</tr>
<tr>
<td>5</td>
<td>277</td>
<td>gtgggggcttcagaaggg</td>
<td>gggagctgccaggccagc</td>
<td>63.2</td>
<td>63.2</td>
</tr>
<tr>
<td>6</td>
<td>242</td>
<td>attttggggtcaccacctg</td>
<td>cctgcagccttcagc</td>
<td>63.6</td>
<td>63.6</td>
</tr>
<tr>
<td>7</td>
<td>287</td>
<td>cccacacgggcacggtggc</td>
<td>tatccagcttcagctg</td>
<td>63.0</td>
<td>63.0</td>
</tr>
<tr>
<td>8</td>
<td>242</td>
<td>cccacacgggcttcagc</td>
<td>cctgcagccttcagc</td>
<td>63.4</td>
<td>63.4</td>
</tr>
<tr>
<td>9</td>
<td>277</td>
<td>gggtatccacccgggagcaagc</td>
<td>cttgagcctcagc</td>
<td>63.0</td>
<td>63.0</td>
</tr>
</tbody>
</table>

Novel SNPs in CYP2D6 Gene

SNP22 (295)

1 minute, annealing for 1 minute, extension at 68°C for 5 minutes, and a final extension at 72°C for 7 minutes. The annealing temperatures for long PCR summarized in Table 1.

All the CYP2D6 specific products, diluted 1:10 in water, were used as a DNA template for a second round PCR of all the CYP2D6 exons. Table 2 lists the primer pairs that were used to amplify CYP2D6 exons. These primers were designed based on the genomic sequence reported in GenBank (M33388). Amplicons were generated with the AmpliTaq Gold PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The thermal profile consisted of denaturation at 95°C for 10 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 7 minutes. Heteroduplexes were generated by thermal cycling as follows: 95°C for 1 minute, followed by a reduction in temperature from 95°C by 45 increments of 1.5°C per minute.

The PCR products were analyzed using the DHPLC system, WAVE® (Transgenic Inc., Omaha, NE, USA). Unpurified PCR samples (5 μL) were separated on a heated C18 reverse phase column (DNASepp®) using 0.1 M triethylammonium acetate (TEAA) in water and 0.1 M TEAA in 25% acetonitrile at a flow rate of 0.9 mL/min. The software provided with the instrument selected the temperature for the heteroduplex separation in the heterozygous CYP2D6 fragment.

Table 2 summarizes the DHPLC running conditions for each amplicon. The linear acetonitrile gradient was adjusted to the retention time of the DNA peak at 4-5 minutes. Homozygous nucleotide exchanges can sometimes be distinguished due to a slight shift in the elution time when compared with the reference. The addition of an approximately equal amount of wild-type DNA to the samples (1:1) prior to the denaturation step enabled homozygous alterations to be detected reliably. This was done routinely for all the samples in order to identify homozygous sequence variations. Therefore, all the samples were first analyzed without mixing with an equal amount of wild-type DNA; subsequently, wild-type DNA was mixed with each sample to detect homozygous variants. The resultant chromatograms were compared with the chromatograms of wild-type DNA.

Both strands of samples with variants detected using DHPLC were analyzed using a CEQ8000® automated DNA sequencer (Beckman-Coulter Inc., Fullerton, CA, USA). We also sequenced all the samples with chromatographic findings that differed from the wild-type DNA in order to establish links between mutations and specific profiles. We sequenced the PCR products by the fluorescent dideoxy termination sequencing using the DTCS DNA Sequencing Kit (Beckman-Coulter Inc.) accordance with the manufacturer’s instructions.

For the haplotype analysis of CYP2D6 variant alleles, the PCR products of entire CYP2D6 genes were
DHPLC chromatograms of exon 5 (A) and exon 8 (B) of human CYP2D6 gene. The elution profiles of heterozygous sequence variants are compared with a reference wild-type DNA chromatogram.

**Fig. 1.** DHPLC chromatograms of exon 5 (A) and exon 8 (B) of human CYP2D6 gene. The elution profiles of heterozygous sequence variants are compared with a reference wild-type DNA chromatogram.

2556C>T
(Thr<sup>261</sup>Ile)

![](image1)

3835A>C
(Lys<sup>404</sup>Gln)

![](image2)

**Fig. 2.** The nucleotide sequences of the CYP2D6 gene in exon 5 and exon 8. Although sequences are shown for sense strands, both strands were sequenced. Arrows indicate the variant nucleotide positions.
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subcloned into a pCR®-XL-TOPO® vector (Invitrogen Co., CA, USA). The clones inserted into the CYP2D6 fragments were sequenced using a CEQ8000® automated DNA sequencer.

Results and Discussion

We found two novel SNPs as follows:
1) SNP: 050301Hiratsuka04; GENE-NAME: CYP2D6; ACCESSION NUMBER: M33388; LENGTH: 25 bases; 5’-AGCACAGGATGAC-3’WCTGGGACCCAGC-3’?
2) SNP: 050301Hiratsuka05; GENE-NAME: CYP2D6; ACCESSION NUMBER: M33388; LENGTH: 25 bases; 5’-TCATCGGTGCTGA-3’WCGGATGAGGCCG-3’?

The DHPLC chromatograms and the electrophoregrams of the novel SNPs are shown in Figs. 1 and 2, respectively. The first SNP was 2556C→T, resulting in an amino acid change of Thr261Ile. Haplotype analysis indicated that 100C→T, 1039C→T, 1661G→C, and 4180G→C existed in the same allele of the CYP2D6 gene (Fig. 3). Of the 286 individuals, one was heterozygous for the 2556C→T SNP, suggesting that the allele frequency was 0.002 in the Japanese population. The second SNP was 3835A→C, resulting in an amino acid change of Lys404Gln. Haplotype analysis indicated that 1661G→C, 2850C→T, and 4180G→C existed in the same allele of the CYP2D6 gene (Fig. 3). Of the 286 individuals, one was heterozygous for the 3835A→C SNP, suggesting that the allele frequency was 0.002 in the Japanese population. The sequences for each sample were obtained from at least two different PCR amplifications.

These novel SNPs are located in the exons of the CYP2D6 gene and result in amino acid substitutions. The Thr261 and Lys404 in CYP2D6 are located in G-helix and K”-helix, respectively.®11-12) These amino acid residues are not mapped in substrate recognition sited, but are conserved in the CYP2D subfamily in mammals.®13) Thus, these amino acid substitutions, Thr261Ile and Lys404Gln, are expected to alter the catalytic properties of the CYP2D6. Further studies are required to elucidate the functional characteristics of these novel variant alleles of the CYP2D6 gene.

In the present study, fourteen CYP2D6 alleles were detected in all the 286 Japanese individuals. The most frequent variant allele was CYP2D6*10, followed by *2, *5, and *21, and their frequencies were observed to be 0.362, 0.112, 0.072, and 0.007, respectively. The most frequent defective allele in the Japanese population is CYP2D6*5, which is associated with the PM phenotype. To date, the non-functional alleles of CYP2D6 that have been observed in Japanese population are CYP2D6*4, *5, *14, *18, *21, and *44. However, none of the 286 individuals analyzed at least by DHPLC method in this study showed the presence of CYP2D6*14, *18, or *44. This discrepancy may be caused by differences in sample sizes among these studies. Soyama et al.®14) have recently reported the detection of five novel alleles, CYP2D6*47, *48, *49, *50, and *51. In the present study, CYP2D6*49 and *50 alleles were also found, with frequencies of 0.003 and 0.002, respectively.

The 23 different genotypes found in this study are listed together with their respective frequencies in Table 3. The most frequent genotypes were CYP2D6*1/*10, *1/
The previous data, the PM frequency increased from the CYP2D6 frequency, by adding the frequencies of these SNPs to the literature. The newly identified SNPs (Thr261Ile and Lys404Gln) affect the CYP2D6 function.

Establish whether the newly identified SNPs (Thr261Ile and Lys404Gln) affect the CYP2D6 function. In conclusion, we found two novel nonsynonymous SNPs of CYP2D6 in Japanese individuals. Further studies are being conducted in our laboratory to establish whether the newly identified SNPs (Thr261Ile and Lys404Gln) affect the CYP2D6 function.

**References**


Novel SNPs in CYP2D6 Gene

SNP26 (299)


