SNP Communication

**Novel Mutations of the CYP2A6 Gene in a Thai Population with Lowered Capacity of Coumarin 7-Hydroxylation**

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**Summary:** We explored genetic polymorphisms in a Thai population which exhibited a low capacity to metabolize coumarin. The following two silent single nucleotide polymorphisms (SNPs) were found:

1) SNP, 020228Kiyotani001; GENE NAME, CYP2A6; ACCESSION NUMBER, NT_011139; LENGTH, 25 base; 5'-AAACTACCTGCAG/TCTGAAACACAGAG-3'.
2) SNP, 020228Kiyotani002; GENE NAME, CYP2A6; ACCESSION NUMBER, NT_011139; LENGTH, 25 base; 5'-AATCCCCACCAC/TTCCTGAATGAG-3'.

These two mutations (G144A and C1245T), which were located in exon 1 and exon 8 of the CYP2A6 gene, were found in two subjects among nine poor metabolizers for coumarin.

**Key words:** P450 2A6; genetic polymorphism; poor metabolizer; coumarin; silent mutation; 7-hydroxycoumarin

**Introduction**

Cytochrome P450 2A6 (CYP2A6) is known as an enzyme responsible for the metabolism of clinically used drugs such as coumarin\(^1\)\(^-\)\(^3\) and tegafur.\(^4,5\) CYP2A6 is also involved in the C-oxidation of nicotine to cotinine\(^6\) as well as in the further conversion of cotinine to different metabolites.\(^7\)

Several genetic polymorphisms in the CYP2A6 gene have been described (see http://www.imm.ki.se/CYPalleles/). In our previous study, it was revealed that the interindividual variation in coumarin 7-hydroxylase activity was associated with the genetic polymorphism of the CYP2A6 gene in 200 Thai subjects.\(^8\) Nine subjects exhibited low capacity to metabolize coumarin. Although there were four subjects of the nine poor metabolizers (PMs) genotyped as homozygous for the CYP2A6*4C allele, the other five subjects were judged as CYP2A6*1A/*1A, CYP2A6*1A/*1B, CYP2A6*1B/*1B or CYP2A6*1A/*4C. In the present study, we sequenced all exons of the CYP2A6 gene of these five Thais. We identified three SNPs in exon 1, exon 5 and exon 8, causing no amino acid substitutions. The silent mutation in exon 5 has been already reported in the GenBank database.

**Materials and Methods**

Details for Thai subjects were described in our previous paper.\(^9\) Informed consent was obtained from every volunteer. This study was approved by the ethics committee of Hokkaido University and Chulalongkorn University.

To ensure specific amplification of exon 1 and a region from exon 7 to exon 8 of the CYP2A6 gene, intronic polymerase chain reaction (PCR) primers were designed (Table 1). The genomic sequence of the complete human CYP2A6 gene has been available in GenBank; accession number NT_011139. The PCR for exon 1 was conducted in a reaction mixture containing 50 ng of genomic DNA, 1×LA-PCR buffer II, 1.2 mM MgCl₂, 0.2 mM dNTPs, 10 pmol of each primer (2A6...
Table 1. Oligonucleotide primers used for amplification and sequencing of CYP2A6 coding exons

<table>
<thead>
<tr>
<th>Primer</th>
<th>5’-3’ Sequence</th>
<th>Location</th>
<th>Amplified exon</th>
</tr>
</thead>
<tbody>
<tr>
<td>2A6 up-0.1kb</td>
<td>5’-ggcatctagtggagggg-3’</td>
<td>5’-Flanking region</td>
<td>Exon 1</td>
</tr>
<tr>
<td>2A6 ex1R</td>
<td>5’-gatctgctgcggtgctg-3’</td>
<td>Intron 1</td>
<td></td>
</tr>
<tr>
<td>2A6 ex7</td>
<td>5’-gtcctgagacctatggcc-3’</td>
<td>Intron 6</td>
<td></td>
</tr>
<tr>
<td>2A6 ex8R</td>
<td>5’-gctgacttcgtggggtg-3’</td>
<td>Intron 8</td>
<td>Exon 7 + 8</td>
</tr>
</tbody>
</table>

Fig. 1. The sequences of the CYP2A6 gene at exon 1 and exon 8. Sequences were determined using ABI PRISM 377 sequencer (Perkin Elmer, CA, USA). The primers for direct sequencing were 2A6 ex1R or 2A6 ex8R as described in Table 1. Sequences of antisense strand are shown. (A) Wild/wild in exon 1. (B) Wild/C96T mutation in exon 1. (C) Wild/wild in exon 8. (D) Wild/G1245A mutation in exon 8. Arrows indicate the substituted nucleotides.

up-0.1 kb and 2A6 ex1R), and 1.0 U LA-Taq DNA polymerase in a final volume of 25 μL. PCR was carried out as follows: initial denaturation at 94°C for 1 min followed by 35 cycles of reactions composed of denaturation at 94°C for 30 s, annealing at 60°C for 20 s, and extension at 72°C for 3 min. The expected size of the PCR product was 440 base pair (bp).

The PCR for a region from exon 7 to exon 8 was conducted in a reaction mixture containing 50 ng of genomic DNA, 1× LA-PCR buffer II, 2.0 mM MgCl₂, 0.2 mM dNTPs, 10 pmol of each primer (2A6 ex7 and 2A6 ex8R), and 1.0 U LA-Taq DNA polymerase in a final volume of 25 μL. PCR was performed under the same condition as that for exon 1 except that the extension time was 1.5 min. The expected size of the PCR product was 926 bp.

Results and Discussion

We investigated the CYP2A6 gene of the five Thai subjects phenotyped as PMs carrying the CYP2A6 gene. We found the following two novel mutations (G144A in exon 1 and C1245T in exon 8) from the subjects previously genotyped as CYP2A6*1A/*1A and CYP2A6*1A/*1B (Fig. 1).

1) SNP, 020228Kiyotani001; GENE NAME, CYP2A6; ACCESSION NUMBER, NT_011139; LENGTH, 25 base; 5’-AACCTACCTGCAG/TCTG-AACACAGAG-3’.

2) SNP, 020228Kiyotani002; GENE NAME, CYP2A6; ACCESSION NUMBER, NT_011139; LENGTH, 25 base; 5’-AATCCCAAGAC/TTCTC-TGAATGAG-3’.
All other sequences of amplified fragments matched completely with the reported sequences of the CYP2A6 gene. These two subjects also had a C771T mutation in exon 5 (reported in GenBank), but had no mutations in all other exons (data not shown). The two subjects had G/A at the position of 144, C/T at the position of 771, and C/T at the position of 1245 (data not shown). However, we did not investigate which allele contained these three mutations. The actual significance of these mutations in two alleles has not yet been clarified. These SNPs would have some linkage to impaired CYP2A6 function.

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References


