SNP Communication

**Novel Nonsynonymous Polymorphisms of the CYP1A1 Gene in Japanese**

Tetsuya Saito1, Mari Egashira1, Kazuma Kiyotani1, Masaki Fujieda1, Hiroshi Yamazaki1, Chikako Kyohara2, Hideo Kunitoh1 and Tetsuya Kamataki1

1Laboratory of Drug Metabolism, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan
2Department of Preventive Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
3National Cancer Center Hospital, Tokyo, Japan

**Summary:** We found five novel nonsynonymous polymorphisms of the human CYP1A1 gene from Japanese individuals. The five single nucleotide polymorphisms (SNP) in exon 7 (2346–2347 ins T, 2414T→A, 2461C→T, 2500C→T and 2546C→G causing premature stop codon, Ile448Asn, Arg464Cys, and Arg477Trp and Pro492Arg, respectively) were as follows:

SNP, 030212Saito001; GENE NAME, CYP1A1; ACCESSION NUMBER, X02612; LENGTH, 25 base; 5′-GTCAACCCATCT–GAGTTCCTACCT-3′.

SNP, 030212Saito002; GENE NAME, CYP1A1; ACCESSION NUMBER, X02612; LENGTH, 25 base; 5′-GTGAGAAGGTGATATATTTGGCAT-3′.

SNP, 030212Saito003; GENE NAME, CYP1A1; ACCESSION NUMBER, X02612; LENGTH, 25 base; 5′-GAGACCTTGGCCGTGTCGAGGTGT-3′.

SNP, 030212Saito004; GENE NAME, CYP1A1; ACCESSION NUMBER, X02612; LENGTH, 25 base; 5′-ATCTGGCTGAACTTGTTGAAATTCA-3′.

SNP, 030212Saito005; GENE NAME, CYP1A1; ACCESSION NUMBER, X02612; LENGTH, 25 base; 5′-TGACATGACCC/GCATCTATGGGT-3′.

**Key words:** P450 1A1; genetic polymorphism; aryl hydrocarbon hydroxylase

**Introduction**

Human cytochrome P450 1A1 (CYP1A1) is an aryl hydrocarbon hydroxylase (AHH) responsible for the bioactivation of various carcinogens such as benzo[a]pyrene.1 Genetically controlled interindividual variation of AHH activity and its inducibility has been reported in wide ethnic groups.2–4 To date, six nonsynonymous polymorphisms of the CYP1A1 gene have been reported: CYP1A1*2 (Ile462Val), CYP1A1*4 (Thr461Asn), CYP1A1*5 (Arg464Ser), CYP1A1*6 (Met331Ile), CYP1A1 variant (Gly45Asp) and CYP1A1 variant (Ile 286Thr) (see http://www.imm.ki.se/CYPalleles/ and http://snp500cancer.nci.nih.gov/home.cfm). In this study, we identified five novel nonsynonymous polymorphisms of the CYP1A1 gene in Japanese. The polymorphisms were 2346–2347 ins T, 2414T→A, 2461C→T, 2500C→T and 2546C→G causing premature stop codon, Ile448Asn, Arg464Cys, and Arg477Trp and Pro492Arg, respectively.

**Materials and Methods**

Detailed information on the subjects was described previously.4,5 Informed consent was obtained from every volunteer. This study was approved by the ethics committee of Hokkaido University. The sequence of the human CYP1A1 gene described in the GenBank (accession number X02612) was used as a reference. The A of ATG start codon was defined as +1. The primers used for the specific amplification and the direct sequencing of all seven exons and exon-intron junctions of the CYP1A1 gene were designed as follows: 1Laboratory of Drug Metabolism, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan
2Department of Preventive Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
3National Cancer Center Hospital, Tokyo, Japan

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To whom all correspondence should be addressed: Dr. Tetsuya Saito, Laboratory of Drug Metabolism, Graduate School of Pharmaceutical Sciences, Hokkaido University, N12W6, Kita-ku, Sapporo, Hokkaido 060-0812, Japan. Tel. & Fax. * +81-11-706-3235, E-mail: tetu@pharm.hokudai.ac.jp

SNP20 (218)
Table 1. Primers used for the specific amplification and direct sequencing analysis of the human CYP1A1 gene

<table>
<thead>
<tr>
<th>Primers</th>
<th>Primer sequence (5’-3’ orientation)</th>
<th>Location</th>
<th>Amplified exon</th>
</tr>
</thead>
<tbody>
<tr>
<td>I462V-S</td>
<td>GCTGCTTGCCCTGTCCTCTAT</td>
<td>intron 6</td>
<td>exon 7</td>
</tr>
<tr>
<td>I462V-A</td>
<td>AGGCATGCTTCAATTGTTAGC</td>
<td>exon 7</td>
<td></td>
</tr>
<tr>
<td>ex1-S</td>
<td>CGAGTCCTGAGGTATGGCTGA</td>
<td>5’-flanking</td>
<td>exon 1</td>
</tr>
<tr>
<td>ex1-A</td>
<td>CCCAGCTTGGCAATCTGTG</td>
<td>intron 1</td>
<td>exon 2</td>
</tr>
<tr>
<td>ex2-S</td>
<td>CCCGACTGTGATGTTCAACA</td>
<td>intron 2</td>
<td>exon 2</td>
</tr>
<tr>
<td>ex3-S</td>
<td>AGAGCCCTGGACAGGAGCAGAG</td>
<td>intron 2</td>
<td>exon 3-7</td>
</tr>
<tr>
<td>ex6-A</td>
<td>GCGAATGCTTCAACAGATAC</td>
<td>exon 7</td>
<td></td>
</tr>
<tr>
<td>ex7-S</td>
<td>AGCTTGGTACACCACATCT</td>
<td>exon 7</td>
<td></td>
</tr>
<tr>
<td>ex7-A</td>
<td>TCTTCCCTCCCCCTACAGTA</td>
<td>intron 7</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. The nucleotide sequences of the human CYP1A1 gene at exon 7 containing 2546C>G polymorphism (Pro492Arg). The sequences are shown for sense strands. Arrows indicate the variant nucleotide positions.

Coding Variants of CYP1A1 SNP21

human CYP1A1 gene are shown in Table 1. PCR for each region of the CYP1A1 gene was conducted in a 25 μL reaction mixture containing 100 ng of genomic DNA, KOD-plus-buffer, 1.0 mM MgSO4, 5.0% DMSO, 0.2 mM dNTPs, 5.0 pmol of each primer and 1.0 U KOD-plus-DNA polymerase. PCR conditions consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 30 s and extension at 68°C for 2 min except for Ile462Val genotyping primer (I462V-S and I462V-A, 68°C for 1 min). Sequences were determined using ABI PRISM 3100 genetic analyzer (Applied Biosystems).

Results and Discussion

During the course of genotyping for a known Ile462Val polymorphism of the human CYP1A1 gene in 261 Japanese individuals, we found a novel nonsynonymous SNP as follows:

SNP, 030212Saito005; GENE NAME, CYP1A1; ACCESSION NUMBER, X02612; LENGTH, 25 base; 5’-TGGACATGACCCGATCTATGGGCT-3’. The SNP was 2546C>G in exon 7 resulting in a amino acid change of Pro492Arg (Fig. 1). Four out of 261 individuals were heterozygous for the SNP, suggesting that the allele frequency was 0.8% in Japanese. Direct sequencing of the entire exons and exon-intron boundaries of the CYP1A1 gene from the four subjects revealed no genetic linkage of the SNP with known CYP1A1 polymorphisms including Mpi1 polymorphism. Then, the SNP was registered as a new CYP1A1 allele, CYP1A1*11.

Additionally, we identified four nonsynonymous variants of the CYP1A1 gene, although only one heterozygote was found for each variant. Identified variants were 2346_2347 ins T, 2414T>A, 2461C>T and 2500C>T causing premature stop codon, Ile462Asn, Arg464Cys and Arg477Trp, respectively (Fig. 2). The allele names of these variants were CYP1A1*7, CYP1A1*8, CYP1A1*9 and CYP1A1*10, respectively. These four variants in addition to 2346_2347 ins T generating premature stop codon, like a known Ile462Val, are closely located in the heme-binding region. Thus, these amino acid substitutions are expected to alter the catalytic activity of CYP1A1.

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Fig. 2. The nucleotide sequences of the human CYP1A1 gene at exon 7 containing the variants found in only one subject. The sequences are shown for sense strands. Arrows indicate the variant nucleotide positions.
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References