SNP Communication

A Novel Single Nucleotide Polymorphism (SNP) of the CYP2C19 Gene in a Japanese Subject with Lowered Capacity of Mephobarbital 4'-Hydroxylation

Jun Morita¹, Kaoru Kobayashi*¹, Atsuko Wanibuchi², Miyuki Kimura², Shin Irie², Takashi Ishizaki¹, and Kan Chiba¹
¹Laboratory of Pharmacology and Toxicology, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan
²Kyushu Pharmacology Research Clinic, Fukuoka, Japan
³Department of Pharmacology and Therapeutics, Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan

Summary: We sequenced all nine exons and exon-intron junctions of the cytochrome P450 2C19 (CYP2C19) gene from a Japanese subject with a lowered capacity of CYP2C19-mediated 4'-hydroxylation after an oral administration of mephobarbital. We found a novel single nucleotide polymorphism (SNP) of CYP2C19 gene as follows: SNP, 040110MoritaJ001; GENENAME: CYP2C19; ACCESSION NUMBER: NT_030059.8; LENGTH: 25 bases; 5'-GAGGGCCTGGCCCATGGAGCTGT-3'. The SNP (168946C→T) induced an amino acid alteration (Arg442Cys) located in exon 9 close to the heme-binding region of CYP2C19, which may result in the decrease in the catalytic properties of CYP2C19. A new allele having this SNP was designated as CYP2C19*16.

Key words: CYP2C19; novel SNP; amino acid substitution; Japanese

Introduction

Mephobarbital is metabolized to 4'-hydroxymephobarbital by CYP2C19 in human liver microsomes. Recently, we investigated the pharmacokinetic disposition and metabolism of mephobarbital in extensive metabolizers and poor metabolizers of CYP2C19 recruited from a Japanese population. Through the study, we found that a heterozygote of defective allele, CYP2C19*2, had a lower capacity for the 4'-hydroxylation of mephobarbital compared with the other heterozygotes. Urinary excretion rate of 4'-hydroxymephobarbital collected in 0 to 24 hours after an oral administration of mephobarbital in the subject was only one third of those in the other heterozygotes of defective alleles, CYP2C19*2 or CYP2C19*3 (Kobayashi et al). Although all of known SNPs of CYP2C19 gene (see http://www.imm.ki.se/CYPalleles/) have been analyzed, none of these mutated alleles were existed in the genomic DNA of this outlier, except for CYP2C19*2. Therefore, we sequenced all nine exons and exon-intron junctions of CYP2C19 gene from the outlier and identified a novel SNP located in exon 9 of CYP2C19 gene and the SNP was also analyzed in 80 Japanese reported herein.

Materials and Methods

Human DNA samples: Genomic DNA was isolated from whole blood of healthy volunteers using GFX genomic Blood DNA Purification kit (Amersham Biosciences, Piscataway, NJ, USA) in accordance with the manufacturer's instructions. Informed consent was obtained, and the study including phenotyping and
Table 1. Primers used for the specific amplification and direct sequencing analysis of CYP2C19 gene

<table>
<thead>
<tr>
<th>amplified region</th>
<th>forward primer (5' to 3')</th>
<th>reverse primer (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon1</td>
<td>AGTGGGCTAGGTTGATGGGCACTT</td>
<td>TCAAGTGTTTTATTTACAATGATTC</td>
</tr>
<tr>
<td>Exons 2 and 3</td>
<td>TAAATATGAAATCTAAATGCAGTTG</td>
<td>GGAAGGCAAGTCCAGAAAGGTCAGTGATA</td>
</tr>
<tr>
<td>Exon 4</td>
<td>TGCCTTTTAAAGGGAGTCACTAGG</td>
<td>CAAATGTACTTCCAGGGCTTG</td>
</tr>
<tr>
<td>Exon 5</td>
<td>CAACCCAGAGCTGGCATATTG</td>
<td>TGAATGCCTACTGGATATTCATGC</td>
</tr>
<tr>
<td>Intron 5</td>
<td>AAAACCTGCTTTTTATGGGAAATG</td>
<td>ATAAACTAAGCTTCTGTTAAACATGT</td>
</tr>
<tr>
<td>Exon 6</td>
<td>AAAACCTGCAACAAACAGGGATG</td>
<td>AAATTTGGGACAGTACAGCTG</td>
</tr>
<tr>
<td>Exon 7</td>
<td>AATTGCTAGAAACAAATGTTGCTTTTTC</td>
<td>AGAGGGTAAACATACCTG</td>
</tr>
<tr>
<td>Exon 8</td>
<td>CCACGTCTATACACACATCGTGA</td>
<td>GAAGGCTGCATGAAATGG</td>
</tr>
<tr>
<td>Exon 9</td>
<td>ATCTACTCATCCTCTATGATCCCG</td>
<td>ATGGGCACTTCAATGTAATATAGA</td>
</tr>
</tbody>
</table>

A) Wild type/wild type

B) Wild type/168946C>T

C) Partial amino acid sequence of CYP2C19

\[
\text{PFSAGKRICVGELAR}^{442} \text{M}
\]

Fig. 1. The nucleotide sequences of the CYP2C19 gene in exon 9 containing 168946C>T polymorphism (Arg442Cys). Although the sequences are shown only for sense strands, both the strands were sequenced. The first A of the translational initiation codon ATG of exon 1 is defined as position +1. A) Wild type/wild type. B) Wild type/168946C>T. C) Partial amino acid sequence of CYP2C19 showing the conserved heme-binding motif (FXXGXRXCXG). Arrows indicate variant nucleotide and amino acid positions. Underlines indicate the conserved heme-binding motif of cytochrome P450.
closely located in the heme-binding region. Thus, the initial denaturation at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 1 min, except that annealing was performed at 65°C for amplification of exon 1. Sequences were determined using Cycle Sequencing with Quick Start Kit (Beckman coulter). The primers used for the PCR amplification and sequencing are shown in Table 1. The SNP was confirmed by the repeated sequence analyses on PCR products generated by new genomic DNA amplification.

**Mephobarbital phenotyping protocol:** Thirty male healthy volunteers received an oral 200-mg dose of racemic mephobarbital (1 tablet of Prominal®, Winthrop Laboratories, Sydney, Australia). Urine samples were collected at 0 to 24 hours. The concentration of 4'-hydroxymephobarbital in urine was measured by the HPLC method. (Kobayashi et al). The genotypic backgrounds of CYP2C19 of thirty male healthy subjects were the nine homo extensive metabolizers (2C19*1/2C19*1), ten hetero extensive metabolizers (2C19*1/2C19*2, 2C19*1/2C19*3) and eleven poor metabolizers (2C19*2/2C19*2, 2C19*2/2C19*3, 2C19*3/2C19*3).

**Results and Discussion**

We found the following novel SNP (168946 C>T) from the outlier subject with a lowered capacity for CYP2C19-mediated 4'-hydroxylation of mephobarbital.

SNP: 040110MoritaJ001; GENENAME: CYP2C19; ACCESSION NUMBER: NT_030059.8; LENGTH: 25 bases; 5'-GAGGGCCCTGGCCC/TGCATGGAGCT-GT-3'.

The SNP was 168946C>T in exon 9 resulting in an amino acid change of Arg442Cys (Fig. 1). The outlier was heterozygous for the Arg442Cys allele. As shown in Fig. 1C, the amino acid substitution (Arg442Cys) is closely located in the heme-binding region. Thus, the novel SNP is expected to decrease the catalytic properties of CYP2C19. In fact, urinary excretion rate of 4'-hydroxymephobarbital collected in 0 to 24 hours after an oral administration of mephobarbital in the subject was lower than those in the other heterozygotes with defective alleles, CYP2C19*2 or CYP2C19*3, or the homozygotes of CYP2C19*1 (3.5% vs. 10.1±1.1 or 10.9±1.5% of dose). However, the rate in the subject was not as low as those in poor metabolizers of CYP2C19 (0.33±0.31% of dose).

Until now, it has been known that the only two defective alleles (i.e., CYP2C19*2 and CYP2C19*3) account for >99% of poor metabolizer alleles in the Japanese population. On the other hand, more than 10 variants (designated as CYP2C19*4 to CYP2C19*15) of CYP2C19 gene have been reported from the other ethnic groups (see http://www.imm.ki.se/CYPalleles/). The novel SNP would be able to explain the poor metabolizer status of CYP2C19 which cannot be explained by CYP2C19*2 and CYP2C19*3 in a Japanese population. However, since this novel SNP was not found in another 80 DNA samples of Japanese subjects, further studies are definitely required for the more exact frequency of the SNP and its functional characteristics in the Japanese and other races.

In conclusion, the novel SNP (Arg442Cys) located in exon 9 of the CYP2C19 gene was found in a Japanese subject with an impaired activity of CYP2C19. This outlier appears to be a heterozygote for CYP2C19*2 and this new SNP, although haplotypes of the subject have not been evaluated. Further studies are undergone to establish whether the newly identified SNP (Arg442Cys) affects the CYP2C19 function in our laboratory.

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**References**


