Detection of Three Genetic Polymorphisms in the 5′-Flanking Region and Intron 1 of Human CYP1A2 in the Japanese Population

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Interindividual variability of the activity of CYP1A2 may be expected to affect cancer susceptibility, since the enzyme is capable of activating several carcinogens. In the present study, we found three new polymorphisms in the 5′-flanking region (CYP1A2/B) and intron 1 (CYP1A2/C and CYP1A2/D) of CYP1A2 in Japanese by using polymerase chain reaction-single strand conformation polymorphism. We developed methods to detect these polymorphisms by polymerase chain reaction-restriction fragment length polymorphism and performed a population study (159 subjects) to estimate the frequencies of the alleles. The frequencies of the CYP1A2/A (adenine), CYP1A2/B (thymine-deleted), CYP1A2/C (guanine) and CYP1A2/D (adenine) variants were 21.1, 42.0, 8.2 and 61.3%, respectively. The results of family study supported the idea that these CYP1A2 genotypes are inherited with an autosomal codominant transmission.

Key words: Polymorphism — PCR-RFLP — Cytochrome P450

Cytochrome P450 (CYP) enzymes play an important role in the metabolism of endogenous and exogenous substrates. Human CYP1A2 has been shown to be responsible for the 3-demethylation of caffeine, the initial major step in the biotransformation of caffeine in humans. CYP1A2 is also known to be involved in the metabolic activation of numerous carcinogens such as 2-aminofluorene, 3-amino-1-methyl-5H-pyrido[4,3-b]-indole (Trp-P-2) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP). Several studies on the CYP1A2-dependent metabolism of caffeine or phenacetin have demonstrated that this enzyme is expressed in human livers at a variety of levels among individuals. Considerable interindividual variability in the activities of CYP1A2-dependent N-oxidation of 2-naphthylamine, 2-acetylaminofluorene and 4-amino-biphenyl has also been noted. The sequence analysis of Japanese DNA samples in our previous study suggested that the considerable variation in the level of CYP1A2 expression was not due to mutation of the exonic, intronic, or 5′-flanking regions. However, our recent study clarified that genetic polymorphism existed in the 5′-flanking region of the human CYP1A2 gene in Japanese subjects. This mutation affects the CYP1A2 inducibility. Further, we discovered three additional polymorphisms of the CYP1A2 gene by using the polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) method in the 5′-flanking region and intron 1 of the CYP1A2 gene (data not shown). The four CYP1A2 mutations are summarized in Fig. 1. CYP1A2/A was already reported by our group. CYP1A2/B, CYP1A2/C and CYP1A2/D are new polymorphic alleles.

In this report, we describe methods to detect the three mutated alleles by PCR-restriction fragment length polymorphism (PCR-RFLP) and we present an estimate of the allele frequencies in a Japanese population. The use of human blood for this study had been approved by the Hokkaido University Ethics Committee. The 159 subjects were all healthy Japanese. Genomic DNA was extracted from peripheral leukocytes with phenol-chloroform, followed by ethanol precipitation. DNA was dissolved in 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA, and stored at 4°C until PCR reactions. Four CYP1A2 genotypes were detected by PCR-RFLPs. The sequences of the primers for PCR are shown in Table I. PCR was performed to detect the CYP1A2/A genotype using primers P1 and P2, to detect the CYP1A2/B genotype using primers P3 and P4, to detect the CYP1A2/C genotype using primers P5 and P6, and to detect the CYP1A2/D genotype using primers P7 and P8. Amplification was performed by 25 cycles of denaturing at 94°C for 1 min, annealing for 1 min, and extension at 72°C for 1 min (CYP1A2/B, CYP1A2/C and CYP1A2/D) or 2

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min (CYP1A2/A). Annealing temperatures were 54°C (CYP1A2/A and CYP1A2/D), 51°C (CYP1A2/B) and 60°C (CYP1A2/C). CYP1A2/A, CYP1A2/B, CYP1A2/C and CYP1A2/D were identified by DdeI-, NdeI-, StuI- and ApaI-RFLP, respectively (Fig. 2). The amplified DNA fragments including the polymorphic site were digested with the restriction enzyme, and subjected to electrophoresis on a 2% agarose gel (CYP1A2/A, CYP1A2/B, CYP1A2/C and CYP1A2/D) or a 10% polyacrylamide gel (CYP1A2/B).

The distribution of the four CYP1A2 genotypes in the healthy Japanese subjects is summarized in Table II. The frequencies of the CYP1A2/A (adenine), CYP1A2/B (thymine-deleted), CYP1A2/C (guanine) and CYP1A2/D (adenine) variants were 21.1, 42.0, 8.2 and 61.3%, respectively. The distribution of CYP1A2/A, CYP1A2/B and CYP1A2/D was in accordance with the frequencies expected when applying the Hardy-Weinberg principle. CYP1A2/C distribution did not follow the Hardy-Weinberg principle, because of the over-representation of G/G genotype.

A family study was performed in 54 subjects from 17 two-generation families to establish whether or not three of the polymorphisms (CYP1A2/B, CYP1A2/C and CYP1A2/D) were inherited. A family study of CYP1A2/A genotype has already been performed by Nakajima et al. The results for other genotypes supported the idea that these CYP1A2 genotypes were inherited with an autosomal codominant transmission (data not shown).

**Table I. Sequences and Locations of Primers Used in PCR-RFLPs of CYP1A2 Alleles**

| Primer Used for | Sequence | Locationa (bp) |
|-----------------|----------|----------------|
| P1 1A2/A sense | 5’-GCT ACA CAT GAT CGA GCT ATA C-3’ | −3097 – −3076 |
| P2 antisense   | 5’-CAG GTC TCT TCA CTG TAA AGT TA-3’ | −2500 – −2522 |
| P3 1A2/B sense | 5’-TGA GCC ATG ATT GTG GCA TA-3b | −1589 – −1571 |
| P4 antisense   | 5’-AGG AGT CTT TAA TAT GGA CCC AG-3’ | −1423 – −1445 |
| P5 1A2/C sense | 5’-AAA GAC GGG GAG CCT GGG CTA GGT GTA GGA G-3b | 126 – 156 |
| P6 antisense   | 5’-AGC CAG GGC CAG GGC TGC CCT TGT GGT AAG-3’ | 294 – 265 |
| P7 1A2/D sense | 5’-CCTT AGA AGT GTA AAC TGA GA-3’ | 615 – 634 |
| P8 antisense   | 5’-GGG TTG AGA TGG AGA CAT TC-3’ | 857 – 838 |

a) Location of primers is numbered according to Quattrochi et al.13) (P1, P2, P3 and P4) and Ikeya et al.14) (P5, P6, P7 and P8).
b) A nucleotide with an underline indicates a base change to incorporate a restriction enzyme site.
Caffeine is metabolized by CYP1A2. Our results have shown that the point mutation from guanine to adenine at base −2964 (CYP1A2/A) causes a significant decrease of CYP1A2 inducibility measured in terms of the rate of caffeine 3-demethylation in Japanese smokers.11) CYP1A2 inducibility measured in terms of the rate of caffeine urinary metabolites. 

Table II. Distribution of CYP1A2 Genotypes in Healthy Japanese Subjects

| Polymorphism | Genotype | Number of subjects (%) |
|--------------|----------|------------------------|
| CYP1A2/A<sup>a</sup> | G/G      | 98 (61.6)              |
|              | G/A      | 55 (34.6)              |
|              | A/A      | 6 (3.8)                |
| CYP1A2/B<sup>b</sup> | T/T      | 53 (33.8)              |
|              | T/del    | 76 (48.4)              |
|              | del/del  | 28 (17.8)              |
| CYP1A2/C<sup>c</sup> | T/T      | 137 (86.2)             |
|              | T/G      | 18 (11.3)              |
|              | G/G      | 4 (2.5)                |
| CYP1A2/D<sup>d</sup> | C/C      | 26 (16.4)              |
|              | C/A      | 71 (44.6)              |
|              | A/A      | 62 (39.0)              |

<sup>a</sup> G, guanine allele; A, adenine variant.
<sup>b</sup> T, thymine allele; del, thymine-deleted variant.
<sup>c</sup> T, thymine allele; G, guanine variant.
<sup>d</sup> C, cytosine allele; A, adenine variant.

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From the results of the in vivo caffeine test, CYP1A2 activity showed not only interindividual differences (14-fold in the Japanese subjects), but also racial differences in the distribution of probit plots. The racial differences in CYP1A2 activity may be due to exposure to different inducers and/or inhibitors in the diet and environment and may also reflect different genetic backgrounds. Our preliminary data suggest that allele frequencies in Caucasians of CYP1A2/A (A variant) and CYP1A2/B (T-deleted variant) were lower than those in Japanese, whereas the frequency of CYP1A2/D (A variant) allele was high in Caucasians when compared with Japanese subjects. Recently, CYP1A2/D polymorphism was reported to exist in Caucasians. This polymorphic allele affected CYP1A2 inducibility, as well as CYP1A2/A polymorphism.

We discovered three new polymorphisms of the CYP1A2 gene. However, further investigation is needed to clarify the mechanism of genetic polymorphism of the human CYP1A2 gene. Our preliminary data indicate that the allele frequency of CYP1A2/A polymorphism in lung cancer patients is higher than in controls. A population study with cancer patients is under way.

A part of this study was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan, and a grant from the Japan Health Science Foundation. This work was supported in part by a Grant-in-Aid from the Ministry of Health and Welfare for the 2nd Term Comprehensive 10-Year Strategy for Cancer Control.

(Received May 19, 1999/Revised August 6, 1999/Accepted August 11, 1999)
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