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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.[1)] CYP1A2 is also known to be involved in the metabolic activation of numerous carcinogens such as 2-aminofluorene, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]-indole (Trp-P-2) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP).[2–4)] 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.[5, 6)] Considerable interindividual variability in the activities of CYP1A2-dependent *N*-oxidation of 2-naphthylamine,[7)] 2-acetylaminofluorene[8)] and 4-aminobiphenyl has also been noted.[9)] 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.[10)] However, our recent study clarified that genetic polymorphism existed in the 5′-flanking region of the human *CYP1A2* gene in Japanese subjects.[11)] 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.[11)] *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.[12)] DNA was dissolved in 10 m*M* Tris-HCl (pH 8.0) containing 1 m*M* 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.[13, 14)] PCR was performed to detect the *CYP1A2*/*A* genotype using primers P1 and P2,[11)] 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 *Dde*I-, *Nde*I-, *Stu*I- and *Apa*I-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*/*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.*[11] The results for other genotypes supported the idea that these *CYP1A2* genotypes were inherited with an autosomal codominant transmission (data not shown).

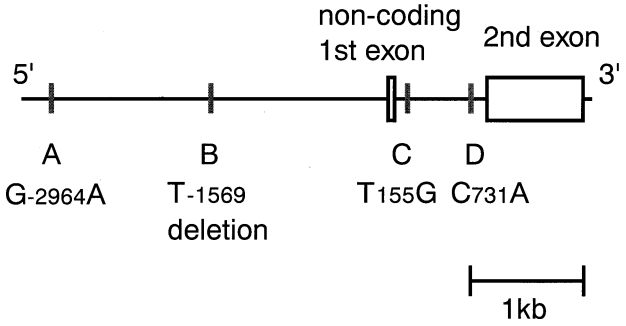

Fig. 1. Location of the four mutations in the *CYP1A2* gene.

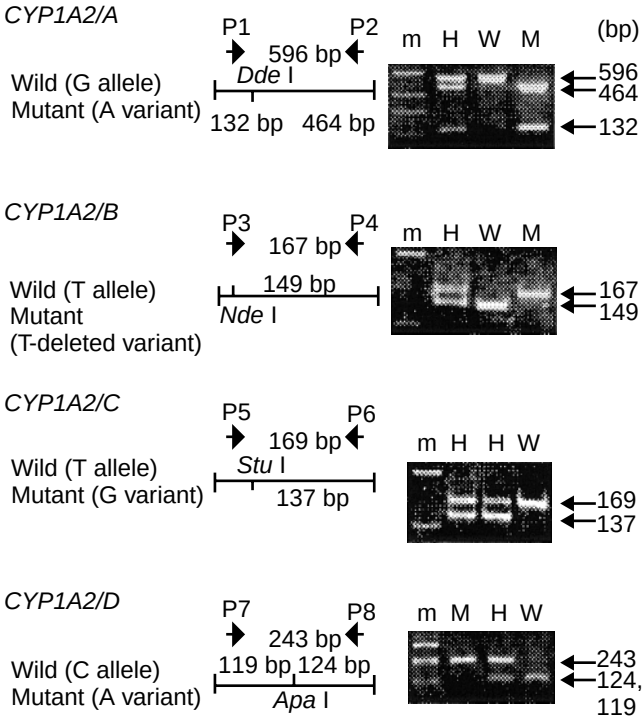

Fig. 2. Detection of *CYP1A2* polymorphism by PCR-RFLP. The length of the PCR product and the RFLP pattern are shown. Arrows indicate the primer. W, H and M mean homozygous wild type, heterozygous and homozygous mutated allele, respectively. A pBR322 vector digested with restriction enzymes, *Eco*RI and *Mva*I, was applied to the gel as a molecular weight standard (m lanes).

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

Primer	Used for		Sequence	Location[a)]
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-3′[b)]	–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-3′[b)]	126 – 156
P6		antisense	5′-AGC CAG GGC CAG GGC TGC CCT TGT GCT AAG-3′	294 – 265
P7	1A2/D	sense	5′-CCC AGA AGT GGA 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.

Table II. Distribution of *CYP1A2* Genotypes in Healthy Japanese Subjects

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

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

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 also mediates the metabolic activation of various carcinogens, including heterocyclic amines.[2–4] The variation of the intensity of CYP1A2 activity could result in increased or decreased capacity to activate carcinogens. Individuals who have a higher capacity to activate carcinogens are expected to be more susceptible to cancer.

Although cigarette smoking has been reported to induce CYP1A2 mRNA and the enzyme activity in the human liver,[10, 15] it has also been reported that smoking history does not account for the observed variability in the expression level and the activity of CYP1A2.[16, 17]

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.[10] 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.[18] 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.

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