Biochemical Pharmacology, Vol. 55, pp. 2039–2042, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(98)00022-7

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SHORT COMMUNICATION

Linkage of Mutant Alleles of *CYP2C18* and *CYP2C19* in a Japanese Population

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ABSTRACT. The coincidence of mutated alleles of *CYP2C18* and *CYP2C19* was studied in 154 Japanese subjects. The mutant alleles of *CYP2C18* studied were $CYP2C18_{m1}$ ($T_{204} \rightarrow A$ substitution in exon 2) and $CYP2C18_{mFR}$ ($A_{-460} \rightarrow T$ substitution in the 5′-flanking region), and those of *CYP2C19* were $CYP2C19_{m1}$ ($G_{689} \rightarrow A$ substitution in exon 5) and $CYP2C19_{m2}$ ($G_{636} \rightarrow A$ substitution in exon 4). They were identified by polymerase chain reaction and restriction fragment length polymorphism. The results indicate that genotypes of $CYP2C18_{m1}$ and $CYP2C18_{mFR}$ are completely coincident with those of $CYP2C19_{m2}$ and $CYP2C19_{m1}$, respectively. The finding suggests that the mutations of *CYP2C18* and *CYP2C19* examined in the present study are very closely linked with each other (i.e. $CYP2C18_{m1}$ vs $CYP2C19_{m2}$ and $CYP2C18_{mFR}$ vs $CYP2C19_{m1}$), at least in a Japanese population. BIOCHEM PHARMACOL **55**;12:2039–2042, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. CYP2C18; CYP2C19; polymorphism; cytochrome P450; Japanese

CYP§ is a superfamily of hemoproteins that play a major role in the oxidative metabolism of various foreign compounds. The human CYP2C subfamily, one of the subfamilies of the CYP2 family, consists of at least four CYP isoforms: CYP2C8, CYP2C9, CYP2C18, and CYP2C19 [1]. CYP2C9 and CYP2C19 metabolize various therapeutic agents including warfarin, tolbutamide, phenytoin, nonsteroidal antiinflammatory drugs, omeprazole, diazepam, and imipramine [2]. In contrast, the role of CYP2C8 and CYP2C18 in drug metabolism remains obscure [2].

Two different mutation events causing a nonfunctional *CYP2C19* allele have been reported [3, 4]. The mutant allele, designated $CYP2C19_{m1}$, contains a single base pair ($G_{689} \rightarrow A$) mutation in exon 5, which creates an aberrant splice site [3]. A second mutant allele, designated $CYP2C19_{m2}$, contains a $G_{636} \rightarrow A$ transition in exon 4, which changes the codon for tryptophan to a premature stop codon [4]. The former mutation accounts for approximately 75–83% of defective *CYP2C19* alleles in Japanese and Caucasians, while the latter mutation is rare in Caucasians but accounts for the remaining defective alleles of *CYP2C19* in Japanese [5].

A mutant allele causing a nonfunctional CYP2C18, which is defined as $CYP2C18_{m1}$, also has been reported [6]. The mutation is a substitution of T_{204} to A in exon 2, which creates a stop codon that yields a protein lacking the heme-binding site. Recently, Tsuneoka *et al.* [7] reported another mutation of *CYP2C18* consisting of A_{-460} to T substitution in the 5′-flanking region (designated tentatively as $CYP2C18_{mFR}$ in this paper). Although the functional property of this mutant allele remains unclear, they reported a complete coincidence in the genotypes of $CYP2C18_{mFR}$ and $CYP2C19_{m1}$, namely that homozygous and heterozygous $CYP2C18_{mFR}$ completely matched with homozygous and heterozygous $CYP2C19_{m1}$, respectively, in all the subjects studied [7].

In the present study, we examined the genotypes of $CYP2C18_{m1}$, $CYP2C18_{mFR}$, $CYP2C19_{m1}$, and $CYP2C19_{m2}$ in 154 unrelated healthy Japanese volunteers and found that the genotypes of $CYP2C18_{m1}$ are completely coincident with those of $CYP2C19_{m2}$, in addition to the coincidence between $CYP2C18_{mFR}$ and $CYP2C19_{m1}$.

MATERIALS AND METHODS

Seven milliliters of venous blood was obtained from 154 unrelated healthy Japanese subjects, and DNA was isolated from peripheral leukocytes with the use of an extraction kit (Genomix, Talent). The *CYP2C19* wild-type gene and the two mutated alleles, $CYP2C19_{m1}$ and $CYP2C19_{m2}$, were identified by a PCR amplification with use of the allele-specific primers and an RFLP method as reported previously [8]. Genomic DNA (200 ng) was amplified in PCR buffer (10 mM of Tris-hydrochloride, pH 8.3, and 50 mM of potassium hydrochloride, 0.01% gelatin) that contained a 200 μM of dNTP mixture (dATP, dCTP, dGTP, and dTTP; Takara Shuzo Co., Ltd.), a 0.2-μM concentration of

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§ *Abbreviations:* CYP, cytochrome P450; PCR, polymerase chain reaction; and RFLP, restriction fragment length polymorphism.

Received 26 August 1997; accepted 8 December 1997.

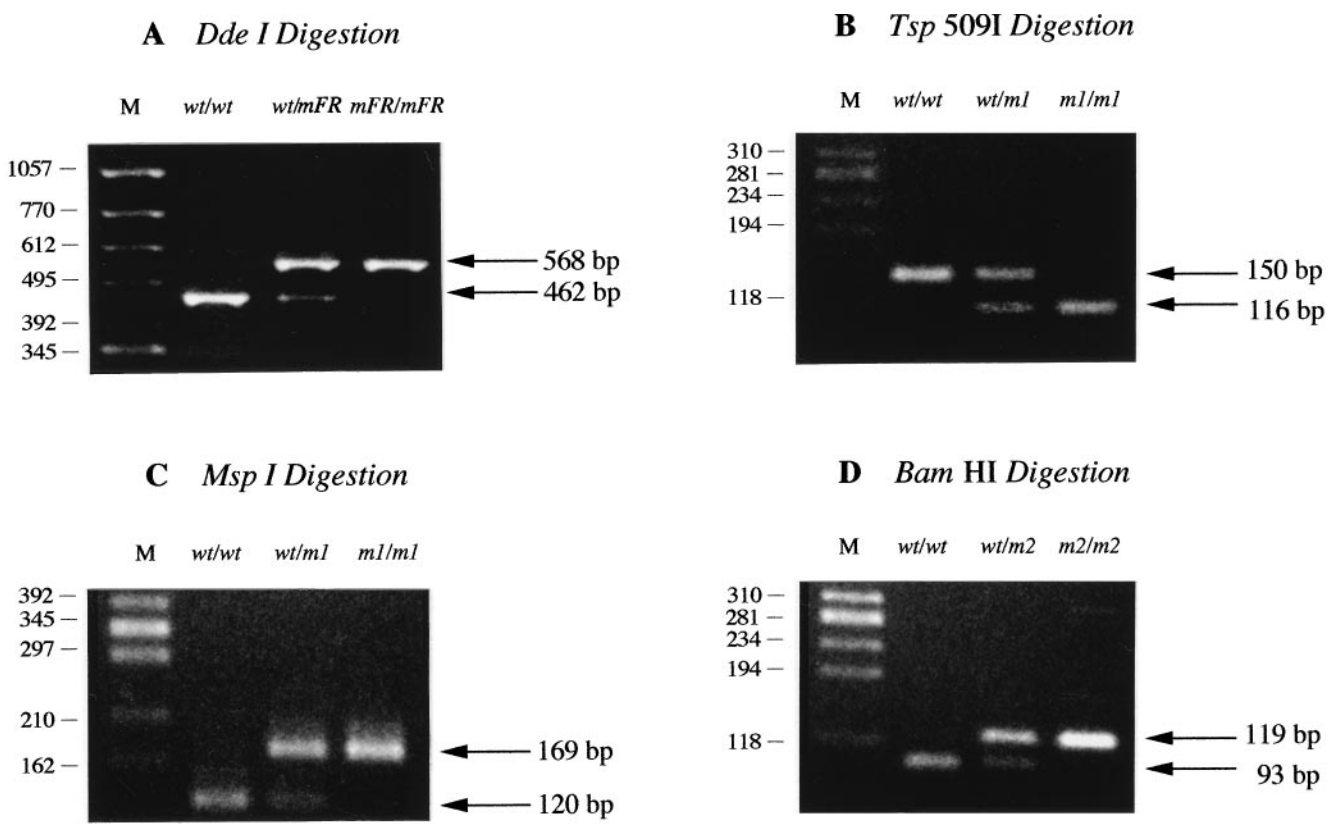

FIG. 1. PCR–RFLP analysis for the mutated alleles of *CYP2C18* (A and B) and *CYP2C19* (C and D) in representative Japanese subjects. Panel A shows the PCR amplification of the 5′-flanking region digested with *Dde*I for *CYP2C18*$_{mFR}$. Panel B shows that of exon 2 digested with *Tsp*509I for *CYP2C18*$_{m1}$. Panel C shows that of exon 5 digested with *Msp*I for *CYP2C19*$_{m1}$. Panel D shows that of exon 4 digested with *Bam*HI for *CYP2C19*$_{m2}$. The predicted base-pair (bp) sizes of the digested DNA fragments for various genotype patterns are shown on the right-hand side. The sizes of the molecular weight markers (M) are shown on the left-hand side.

PCR primers, 1.25 units of AmpliTaq DNA polymerase (Hoffmann-La Roche, Ltd.), and 1.5 mM of magnesium chloride. Amplification was performed with a Perkin Elmer thermocycler, for 40 cycles consisting of denaturation at 94° for 1 min, annealing at 57° for 1 min, and extension at 72° for 2 min. An initial denaturation step at 94° for 5 min and a final extension step at 72° for 5 min were also performed. Restriction enzyme cleavage was conducted at 37° for 1 hr after the addition of 25 units of *Msp*I for *CYP2C19*$_{m1}$ and 25 units of *Bam*HI for *CYP2C19*$_{m2}$. The digested PCR products were analyzed on 3% agarose gels and stained with ethidium bromide.

The *CYP2C18* wild-type gene and the two mutated alleles, *CYP2C18*$_{m1}$ and *CYP2C18*$_{mFR}$, were identified by PCR methods as described by Komai *et al.* [6] and Tsuneoka *et al.* [7], respectively. Genomic DNA (200 ng) was amplified as described above, except that PCR cycles were changed from 40 to 30 for *CYP2C18*$_{m1}$ and annealing time was changed from 1 to 2 min for *CYP2C18*$_{mFR}$. PCR products were digested with each of the restriction enzymes (i.e. *Tsp*509I for *CYP2C18*$_{m1}$, and *Dde*I for *CYP2C18*$_{mFR}$) at the appropriate temperature for 2 hr. The digested PCR products were analyzed on 3% agarose gels and stained with ethidium bromide.

RESULTS AND DISCUSSION

The results of PCR–RFLP analysis for the mutated alleles of *CYP2C18* and *CYP2C19* in representative subjects are shown in Fig. 1. Six different allelic band patterns were observed for both *CYP2C18* and *CYP2C19*.

The results of the analysis of 154 samples are listed in Table 1. As shown in this table, the genotypes of *CYP2C18*$_{mFR}$ were completely coincident with those of *CYP2C19*$_{m1}$, agreeing with the report of Tsuneoka *et al.* [7]. Unexpectedly, the genotypes of *CYP2C18*$_{m1}$ were also found to be completely coincident with those of *CYP2C19*$_{m2}$. The findings indicate that *CYP2C18*$_{m1}$ and *CYP2C18*$_{mFR}$ are very closely linked with *CYP2C19*$_{m2}$ and *CYP2C19*$_{m1}$, respectively.

The precise mechanism of the linkages is unknown; however, it appears to reflect that the *CYP2C18* and *CYP2C19* genes are closely located on the same chromosome. In fact, Gray *et al.* [9] reported that *CYP2C* genes are located together on the chromosome 10q24 in the order of *CYP2C8*, *CYP2C9*, *CYP2C19*, and *CYP2C18* from the upstream to downstream direction. Therefore, the complete linkages between the mutated alleles of *CYP2C18* and *CYP2C19* appear to be derived from the low probability of

TABLE 1. Genotype distribution of $CYP2C18_{m1}$, $CYP2C18_{mFR}$, $CYP2C19_{m1}$, and $CYP2C19_{m2}$ in 154 Japanese subjects

Genotypes of *CYP2C18*	Genotypes of *CYP2C19*					
	wt/wt	*wt/m1*	*wt/m2*	*m1/m2*	*m1/m1*	*m2/m2*
wt/wt	44	—	—	—	—	—
wt/mFR	—	57	—	—	—	—
wt/m1	—	—	22	—	—	—
m1/mFR	—	—	—	17	—	—
mFR/mFR	—	—	—	—	11	—
m1/m1	—	—	—	—	—	3

Abbreviations: *wt*, wild-type; *m1*, *CYP2C19* mutation in exon 5 or *CYP2C18* mutation in exon 2; *m2*, *CYP2C19* mutation in exon 4; and *mFR*, *CYP2C18* mutation in the 5′-flanking region.

crossing-over between $CYP2C18_{mFR}$ and $CYP2C19_{m1}$, and between $CYP2C18_{m1}$ and $CYP2C19_{m2}$.

One may speculate that two sets of the mutations (i.e. $CYP2C18_{mFR}$/$CYP2C19_{m1}$ and $CYP2C18_{m1}$/$CYP2C19_{m2}$) are derived from the gene duplication of *CYP2C19* during the course of evolution. However, the probability appears to be low, because the mutations are not located on the same exon or region of *CYP2C19* and *CYP2C18* in either set of the mutations: the mutation of $CYP2C18_{mFR}$ is A to T substitution at 460 in the 5′-flanking region and that of $CYP2C19_{m1}$ is G to A at 689 in exon 5; the mutation of $CYP2C18_{m1}$ is T to A at 204 in exon 2 and that of $CYP2C19_{m2}$ is G to A at 636 in exon 4.

The present findings suggest that a subject with mutated alleles of $CYP2C19_{m2}$ or $CYP2C18_{m1}$ has a double defect in CYP2C19 and CYP2C18, because both mutations create stop codons and yield nonfunctional premature proteins [4, 6]. On the other hand, whether a subject with mutated alleles, $CYP2C19_{m1}$ or $CYP2C18_{mFR}$, also has a double defect of CYP2C19 and CYP2C18 is unknown, since the functional property of $CYP2C18_{mFR}$ has not been clarified yet [7].

Clinical significance of the linkage of $CYP2C18_{m1}$ and $CYP2C19_{m2}$ is obscure at the present moment. This is because a limited number of drugs have been reported as substrates of CYP2C18, although CYP2C19 metabolizes various clinically used drugs [2]. Moreover, the activity of CYP2C18 for CYP2C19 substrates appears to be low. For example, *CYP2C18* metabolizes racemic mephenytoin, although its turnover number is much lower than that of CYP2C19 [1, 10]. CYP2C18 also catalyzes N-demethylation of diazepam; however, the contribution of the enzyme for the overall N-demethylation activity in human liver microsomes is negligible [11]. Therefore, the clinical significance of the linkage between $CYP2C19_{m2}$ and $CYP2C18_{m1}$ remains uncertain at the present time.

The frequency of $CYP2C18_{mFR}$ or $CYP2C19_{m1}$ in our population calculated from Table 1 was 31.2%, which is not very different from the values reported previously: 21.4% for $CYP2C18_{mFR}$ [7], and 23–29% for $CYP2C19_{m1}$ [3, 8]. The frequency of $CYP2C18_{m1}$ or $CYP2C19_{m2}$ calculated from Table 1 was 14.6%, which is similar to the frequencies of $CYP2C19_{m2}$ reported previously (10.4 to 13.2%) [4, 8]. However, it is approximately half of the value of $CYP2C18_{m1}$ reported previously (27.5%) [6]. Although the reason for the difference is unknown, it may be derived from the small number of subjects employed in the previous study (N = 40) [6].

In conclusion, the present study showed complete linkages between $CYP2C18_{m1}$ and $CYP2C19_{m2}$, and $CYP2C18_{mFR}$ and $CYP2C19_{m1}$ in a Japanese population. However, the clinical significance of the linkages remains unclear at the present time; further study will be required when endogenous or exogenous substrates metabolized efficiently by CYP2C18 are found.

This work was supported by grants-in-aid from the Ministry of Human Health and Welfare, and Drug Innovation Science Project (1-2-7).

References

1. Romkes M, Faletto MB, Blaisdell JA, Raucy JL and Goldstein JA, Cloning and expression of complementary DNAs for multiple members of the human cytochrome P450IIC subfamily. *Biochemistry* **30:** 3247–3255, 1991.
2. Goldstein JA and de Morais SMF, Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics* **4:** 285–299, 1994.
3. de Morais SMF, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA and Goldstein JA, The major genetic defect responsible for the polymorphism of *S*-mephenytoin in humans. *J Biol Chem* **269:** 15419–15422, 1994.
4. de Morais SMF, Wilkinson GR, Blaisdell J, Meyer UA, Nakamura K and Goldstein JA, Identification of a new genetic defect responsible for the polymorphism of (*S*)-mephenytoin metabolism in Japanese. *Mol Pharmacol* **46:** 594–598, 1994.
5. Goldstein JA, Ishizaki T, Chiba K, Morais SMF, Bell D, Krahn PM and Evans DAP, Frequencies of defective *CYP2C19* alleles responsible for the mephenytoin poor metabolizer phenotype in various Oriental, Caucasian, Saudi Arabian and American black populations. *Pharmacogenetics* **7:** 59–64, 1997.
6. Komai K, Sumida K, Kaneko H and Nakatsuka I, Identification of a new non-functional CYP2C18 allele in Japanese: Substitution of T_{204} to A in exon2 generates a premature stop codon. *Pharmacogenetics* **6:** 117–119, 1996.
7. Tsuneoka Y, Matsuo Y, Okuyama E, Watanabe Y and Ichikawa Y, Genetic analysis of the cytochrome P-450IIC18 (CYP2C18) gene and a novel member of the CYP2C subfamily. *FEBS Lett* **384:** 281–284, 1996.
8. Kubota T, Chiba K and Ishizaki T, Genotyping of *S*-mephen-

ytoin 4′-hydroxylation in an extended Japanese population. *Clin Pharmacol Ther* **60:** 661–666, 1996.
9. Gray IC, Nobile C, Muresu R, Ford S and Spurr NK, A 2.4-megabase physical map spanning the *CYP2C* gene cluster on chromosome 10q24. *Genomics* **28:** 328–332, 1995.
10. Goldstein JA, Falleto MB, Romkes-Sparks M, Sullivian T, Kitareewan S, Raucy JL, Lasker JM and Ghanayem BI, Evidence that CYP2C19 is the major (*S*)-mephenytoin 4′-hydroxylase in humans. *Biochemistry* **33:** 1743–1752, 1994.
11. Jung F, Richardson TH, Raucy JL and Johnson EF, Diazepam metabolism by cDNA-expressed human 2C P450s. Identification of P4502C18 and P4502C19 as low K_m diazepam *N*-demethylases. *Drug Metab Dispos* **25:** 133–139, 1997.