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Individual difference in drug metabolism and disposition: Toxicological significance of genotypes and phenotypes of *S*-mephenytoin 4′-hydroxylase (CYP2C19)

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Abstract

We examined the relationship between the genetic polymorphism of CYP2C19 and metabolism of omeprazole in order to assess the severity and to predict the outcome of poisoning for forensic and clinical toxicology. The genotypes observed were *CYP2C19*1A* (*wild type*), *CYP2C19*2* (*m1*), and *CYP2C19*3* (*m2*). The omeprazole hydroxylation index of the wild-type was −1.15, whereas for the hetero-type it was −0.78, and homo-mutated type 1.22. The genotype of CYP2C19 correlated with the phenotype. The present results proved that genotyping assays of drug metabolizing enzymes are of great value in toxicological significance.

Keywords: CYP2C19; Genotyping; Phenotyping; Polymorphism; Toxicogenetics

1. Introduction

The 4′-hydroxylation of *S*-mephenytoin has been shown to be mediated by CYP2C19. It is also important in the metabolism of a number of related hydantoins and barbiturates, as well as of structurally dissimilar drugs such as omeprazole, proguanil, and citalopram. As a result, large interphenotypic differences occur in the disposition of these drugs, which

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may affect their toxicity and efficacy. Therefore, we examined the relationship between the genetic polymorphism of CYP2C19 and the metabolism of omeprazole in order to assess the severity and to predict the outcome of poisoning for forensic and clinical toxicology.

2. Materials and methods

In this study, we prepared DNA samples from the blood of unrelated healthy Japanese, and developed a rapid and simple genotyping method using a polymerase chain reaction (PCR)-based restriction fragment length polymorphism analysis (Fig. 1). Genotyping

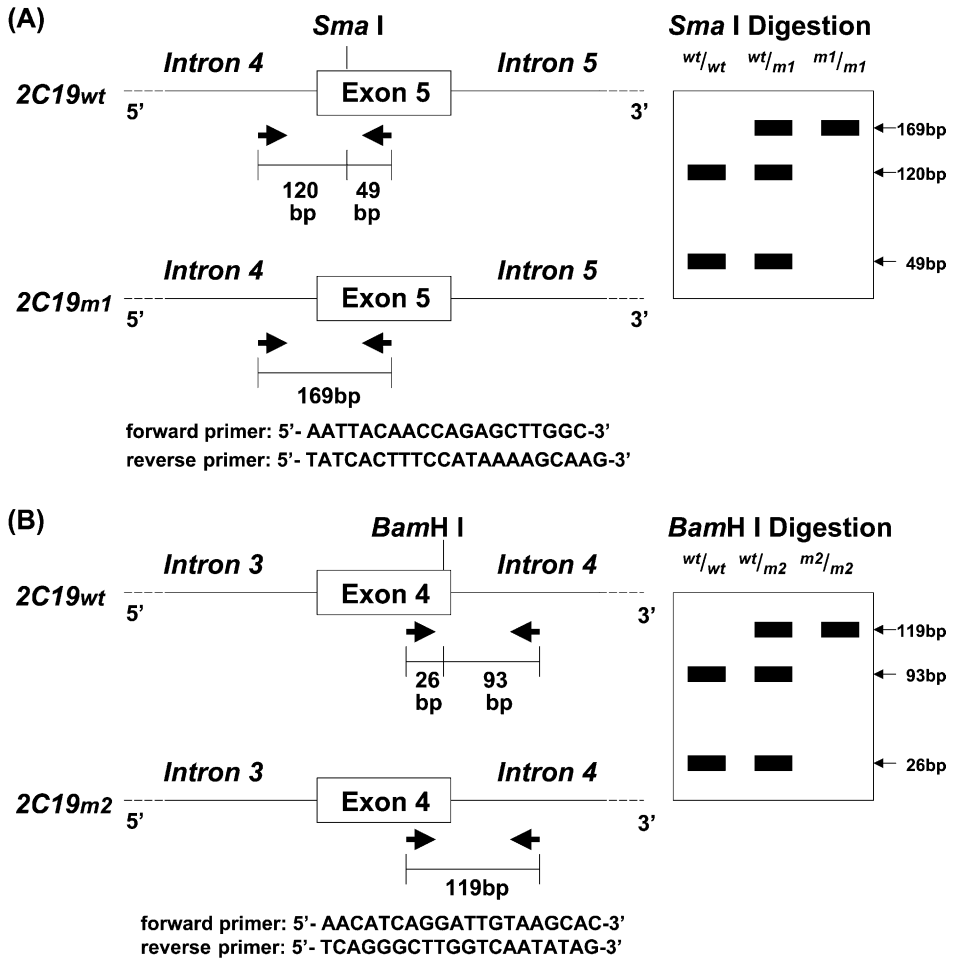

Fig. 1. Strategy used to genotype genomic DNA from human blood, utilizing PCR amplification of exon 5 followed by *Sma*I digestion (*CYP2C19m1*) (A) and amplification of exon 4 followed by *Bam*HI digestion (*CYP2C19m2*) (B). The predicted sizes of the digested DNA fragments for the various genotypes are shown.

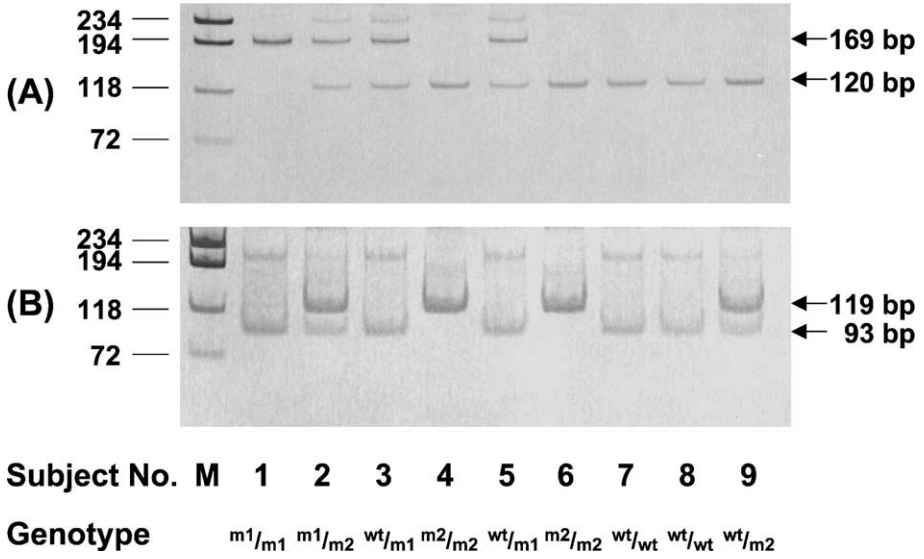

Fig. 2. PCR analysis of exon 5 (A) and exon 4 (B) for the *CYP2C19* gene. A shows the PCR amplification of exon 5 digested with *Sma*I for *CYP2C19m1*. B shows the PCR amplification of exon 4 digested with *Bam*HI for *CYP2C19m2*. The predicted sizes of the digested DNA fragments for the various genotypes are shown on the right. The sizes of the molecular weight markers (M) are shown on the left. PCR products from DNA in individuals with the wild-type (*wt*) allele(s) are cleaved by the restriction enzyme (Subject Nos. 7 and 8), whereas those in homozygous individuals with the mutation lack the *Sma*I or *Bam*HI site and show a single band (Subject Nos. 1, 4 and 6). The predicted sizes of the digested DNA fragments for heterozygous individuals are shown (Subject Nos. 2, 3, 5 and 9).

procedures for the identification of CYP2C19 were performed by PCR amplification with use of the allele-specific primers described by de Morais et al. [1,2] and Kubota et al. [3] with minor modifications. PCR products were digested with the restriction enzymes, and were analyzed by polyacrylamide gel electrophoresis. Furthermore, CYP2C19 phenotypes

Table 1
Correlation between CYP2C19 genotype and phenotype

Genotype[a]	Phenotype[b]	Index[c]
wt/wt	EM	−1.15
wt/m1	EM	−0.78
wt/m2	EM	−0.93
m1/m1	PM	1.22
m1/m2	PM	0.86
m2/m2	PM	1.74

[a] *wt*: *Wild-type*, *m1*: *CYP2C19* mutation in exon 5, *m2*: *CYP2C19* mutation in exon 4.

[b] CYP2C19 phenotype was determined by measuring omeprazole and metabolite concentrations (EM: extensive metabolizer, PM: poor metabolizer).

[c] Omeprazole hydroxylation index was expressed as $\log^{10}$ [omeprazole/5′ -hydroxyomeprazole] in serum collected 2 h after omeprazole (20 mg) ingestion.

were determined by measuring omeprazole and hydroxyomeprazole concentrations in the serum, collected 2 h after omeprazole ingestion, by high-performance liquid chromatography described by Marinac et al. [4] with minor modifications.

3. Results and discussion

The genotypes observed were *CYP2C19*1A* (*wild type: wt*), *CYP2C19*2* (*m1*), and *CYP2C19*3* (*m2*) (Fig. 2). As shown in Table 1, the omeprazole hydroxylation index of wild-type was −1.15, whereas that of the hetero-type was −0.78, and homo-mutated type 1.22. The genotype of CYP2C19 correlated with the phenotype. These results proved that genotyping assays of drug metabolizing enzymes could play a more important role in assessing the severity and in predicting the outcome of poisoning for forensic and clinical toxicology.

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