Limited frequency of the *CYP2C19**17 allele and its minor role in a Japanese population

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- A novel *CYP2C19* gene variant, *CYP2C19**17, is associated with increased metabolic activity.
- Ethnic differences in the frequency of the variant allele have been reported. However, the frequency of the *CYP2C19**17 allele has not been studied in the Japanese population.

WHAT THIS STUDY ADDS

- In a population of 265 healthy Japanese subjects, a low frequency (1.3%) of the *CYP2C19**17 allele was observed.
- The limited frequency of the *17 allele and the absence of a subject homozygous for *17 indicated that *CYP2C19**17 would play a minor role in a Japanese population.

AIMS

We investigated the *CYP2C19**17 allelic frequency in Japanese subjects, and evaluated whether *CYP2C19**17 is an important determinant of interindividual variability of *CYP2C19* activity.

METHODS

We enrolled 265 subjects to determine their *CYP2C19* genotype and plasma metabolic ratio following a single dose of 40 mg omeprazole.

RESULTS

Seven subjects heterozygous for *CYP2C19**17 and no *17/*17 subjects resulted in the *CYP2C19**17 frequency being 1.3%. These heterozygotes had moderate metabolic activities when compared with the metabolic ratio of the other subjects.

CONCLUSIONS

The low frequency of *CYP2C19**17 and the absence of *17/*17 indicates that *CYP2C19**17 plays a minor role in the Japanese population.

Introduction

Cytochrome P450 (CYP) 2C19 metabolizes a wide variety of drugs, such as the proton pump inhibitors (PPIs) omeprazole and lansoprazole, and the anticonvulsant S-mephenytoin [1–3]. A marked interindividual difference has been reported in *CYP2C19* activity and the bimodal distribution of its activity is known as a genetic polymorphism. A subject without the *CYP2C19* activity is called a poor metabolizer (PM) of *CYP2C19* [4], and ethnic

difference is noted in its frequency [5]. A *CYP2C19* PM subject carries two defective *CYP2C19* alleles, and the frequency of mutant alleles causing *CYP2C19* PM also shows ethnic differences [5].

A recent study reported a novel *CYP2C19* gene variant (*CYP2C19**17, –3402C > T and –806C > T), associated with increased gene transcription and thus ultrarapid metabolism of *CYP2C19* substrates [6]. In the study of Sim *et al.* [6] the allelic frequency of *CYP2C19**17 was different between Swedish and Chinese subjects being 18% and

4%, respectively [6], and their findings and previous studies about the frequency of mutant alleles suggest that the frequency of *CYP2C19**17 in a Japanese population would be different from that in a Swedish or a Chinese population. In the present study, therefore, we investigated the allelic frequency of *CYP2C19**17 in 265 Japanese subjects, and evaluated whether *CYP2C19**17 is an important determinant of interindividual variability of *CYP2C19* activity among them.

Methods

Two hundred and sixty-five unrelated healthy Japanese volunteers (183 men and 82 women) were enrolled in this study after giving their written informed consent. They were 5th year students (with a mean age of 25.2 years, ranging from 22 to 37 years and a mean body weight of 61.9 kg ranging from 42 to 100 kg) of Hirosaki University School of Medicine from 2004 to 2006, and they had no history of significant medical illness or hypersensitivity to any drugs. The protocol of the present study was approved by the ethics committee of Hirosaki University School of Medicine.

Each subject was administered two tablets of 20 mg omeprazole (Merck Ltd, Osaka, Japan) with 100 ml of tap water at 09.00 h (1–2 h after eating breakfast), and a blood sample (10 ml) for genotyping and phenotyping was obtained at 13.00–14.00 h (4–5 h after administering omeprazole). They did not take any medication or fruit juices for at least 7 days before the administration of omeprazole.

For *CYP2C19* genotyping, genomic DNA was extracted from the venous blood using an extraction kit (AquaPure Genomic DNA Blood Kit, BIO-RAD, USA). The defective mutation alleles for *CYP2C19*, *CYP2C19**3 and *CYP2C19**2, were identified using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay [7]. Genotyping of the *CYP2C19**17 allele was analyzed by PCR-RFLP (–3402C > T) and allele-specific amplification methods (–806C > T) described by Sim *et al.* [6]. A subject with two defective mutation alleles was categorized as a PM, and a subject without or with one defective mutation allele as an extensive metabolizer (EM).

Plasma concentrations of omeprazole and 5-hydroxyomeprazole were quantified using an HPLC method developed in our laboratory [8]. The limits of quantification were 3 ng ml^{–1} for both omeprazole and 5-hydroxyomeprazole, and the intra- and interday coefficient variations were less than 5.1 and 6.6% for omeprazole (range 4–1600 ng ml^{–1}) and 4.6 and 5.0% for 5-hydroxyomeprazole (range 4–400 ng ml^{–1}), respectively [8]. The plasma omeprazole metabolic ratio (MR) (omeprazole : 5-hydroxyomeprazole) 4–5 h after drug intake was used as a measure of *CYP2C19* activity.

Table 1

CYP2C19 genotype frequencies and metabolic ratio of omeprazole in 265 healthy Japanese volunteers

	Genotype	Number (% of total)	Metabolic ratio (95% CI interval)
EM (n = 217)	*1/*1	94 (35.5)	2.18 ± 2.24 (1.71, 2.65)
	*1/*17	3 (1.1)	0.87 ± 0.25 (0.24, 1.49)
	*1/*2 or *1/*3	116 (43.8)	3.97 ± 3.15 (3.38, 4.55)
	*17/*2 or *17/*3	4 (1.5)	3.85 ± 2.70 (–0.45, 8.15)
PM (n = 48)	*2/*2, *2/*3 or *3/*3	48 (18.8)	32.32 ± 14.55 (28.00, 36.64)

EM, extensive metabolizer; PM, poor metabolizer.

Results

In our 265 Japanese subjects comprising 217 EMs and 48 PMs of *CYP2C19* (Table 1), seven subjects were heterozygous for *CYP2C19**17 and none was homozygous, resulting in the frequencies of *CYP2C19**1, *2, *3 and *17 being 57.9%, 27.9%, 12.8% and 1.3%, respectively.

In the seven subjects heterozygous for *CYP2C19**17, complete linkage existed between the –3402C > T and –806C > T mutations, and both –3402C > T and –806C > T mutations were absent in the *CYP2C19**2 or *CYP2C19**3 allele. These seven subjects showed moderate metabolic activities of *CYP2C19* (Table 1), with the lowest MR among three subjects with *1/*17 or four subjects with *17/*2 or *17/*3 in 12th or 22nd place when compared with those with *1/*1 or *1/*2 or *1/*3, respectively.

Discussion

We investigated the frequency of *CYP2C19**17 in our 265 healthy Japanese subjects, and evaluated whether *CYP2C19**17 is an important determinant of interindividual variability of *CYP2C19* activity among them. A novel *CYP2C19* gene variant (*CYP2C19**17) was reported to have ultrarapid metabolic activity of *CYP2C19*, and ethnic differences were noted in the allelic frequency of *CYP2C19**17 [6]. In our Japanese subjects, the frequencies of *CYP2C19**1, *2, *3 and *17 were 57.9%, 27.9%, 12.8% and 1.3%, respectively. Since the *17 allele was previously genotyped as *CYP2C19**1, these allelic frequencies were in line with other published results [5]. The *17 allele frequency in the Japanese subjects was 1.3% of *CYP2C19* alleles, which is lower than the *17 frequency in Chinese individuals (4%) [6]. This difference is not unusual because some differences have been reported in *CYP2C19* mutant allele frequencies in Asian populations: 39.9% and 6.1% for *2 and *3, respec-

tively, in Chinese subjects [5]; 32% and 5.5% for *2 and *3, respectively, in a Chinese-Taiwanese population [9]; 20.9% and 11.7% for *2 and *3, respectively, in a Korean population [5].

A marked variation in the CYP2C19 activity and lower frequency of the *17 allele implied that the extent of linkage of -3402C > T with the -806C > T mutation affecting gene transcription activity [6] might be different in our Japanese subjects. However, complete linkage existed between the -3402C > T and -806C > T mutations in our Japanese subjects as reported in people of European and Ethiopian descent [6]. The low frequency of CYP2C19-806C > T mutation reported to be 0.8% in a Japanese population [10] was found to be in reasonable agreement with our present findings. In the CYP2C19*2 or *3 allele of our subjects, both -3402C > T and -806C > T mutations were absent, as reported previously [6].

Although the median omeprazole MR of the Swedish *1/*17 subjects was significantly less than that of the *1/*1 subjects [6], our three *1/*17 subjects had moderate metabolic activities compared with the EMs without the *2 or *3 allele, and similar findings were noted in our EMs with one of *2 and *3 alleles. In the present study, the metabolic activity of CYP2C19 was determined by the metabolic ratio of a single point plasma sample 4–5 h after administration of 40 mg omeprazole after breakfast. Since the omeprazole metabolic ratio in a single plasma sample 2–3 h postdose after an overnight fast is proposed as an appropriate phenotyping procedure [11], the differences in the sampling procedure could affect the ratio. However, the limited frequency of the *17 allele and the absence of a subject homozygous for *17 would suggest that the allele is not an important determinant of intersubject variability of CYP2C19 activity in a Japanese population.

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