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Genetic Polymorphisms of Cytochrome P450 2C19 in Functional Dyspeptic Patients Treated with Cimetidine

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Inter-individual pharmacokinetic variation of H_2-receptor antagonist is related to genetic polymorphism of *CYP2C19*. We investigated the frequency of *CYP2C19* genetic polymorphism and the treatment duration of cimetidine by *CYP2C19* genotypes in functional dyspeptic patients without definite causes who were treated with cimetidine in Korea. One hundred subjects with functional dyspepsia participated in this study from March 1, 2010 to June 30, 2011. They were tested by upper gastrointestinal endoscopy and treated for their dyspepsia with cimetidine. The single nucleotide polymorphisms (SNPs) of *CYP2C19* were genotyped using the Seeplex *CYP2C19* ACE Genotyping system. There were no significant differences in the demographic, clinical, or laboratory findings among the *CYP2C19* subgroups which are wild type homozygote (W/W), heterozygote (W/V), and variant homozygote (V/V). The frequencies of *CYP2C19* subgroups were 33 (33%) in W/W, 49 (49%) in W/V, and 18 (18%) in V/V, respectively. The mean duration of cimetidine treatment (in weeks) was the shortest in the V/V among the *CYP2C19* genotypes (W/W: 5.1±1.5, W/V: 4.0±1.7, V/V: 2.1±0.7; $p < 0.001$). This study can also act as a basis for further investigation to identify the underlying genetic, epigenetic, or environmental factors in *CYP2C19* enzyme activity.

Key Words: Cimetidine, *CYP2C19*, Dyspepsia

INTRODUCTION

Dyspepsia is a symptom of the upper abdomen in which patients experience sensations of fullness, bloating, or early satiety [1]. Functional dyspepsia is diagnosed when a specific cause is not found, although the disease may be multifactorial. The treatment of functional dyspepsia must be empiric since a definitive diagnosis is rare. H_2-receptor antagonists (H_2RAs) is commonly prescribed for the treatment of functional dyspepsia in Korea because those are cheaper than proton pump inhibitors (PPIs).

Although many drugs are primarily metabolized by the cytochrome P450s (CYPs), drug metabolism by the CYPs is difficult to study because of many CYPs members and their diverse substrate array [2]. Cimetidine (N-cyano-N'-methyI-N"-[2-[[(5-methyl-1H-imidazol-4-yl)methyl]thio]ethyl]guanidine), a H_2RA, is metabolized mainly by cytochrome P450 2C19 (*CYP2C19*) [3]. The *CYP2C19* genetic polymorphism can influence metabolic activity of the subsequent enzymes [4]. Consequently, it increases the level of exposure in the poor metabolizers (PMs) group enhancing drug effect. The prevalence of PMs with *CYP2C19* enzyme polymorphisms is 2～6% of Caucasians, 15～20% of Japanese, and 10～20% of Africans [5].

This study's goal was to investigate the frequency of *CYP2C19* genetic polymorphism and the treatment duration of cimetidine by *CYP2C19* genotypes in Korean functional dyspeptic patients who were treated by cimetidine and where there was no definite cause.

METHODS

Subjects and study protocol

This study (IRB No. 10-04) was approved by the Institutional Review Board of the Kosin University Gospel Hospital, Busan, Korea, and was performed in accordance with the Helsinki Declaration.

One hundred subjects gave informed written consent and participated in this study from March 1, 2010 to June 30, 2011. Patients did not have any 'alarm' symptoms, including progressive unintentional weight loss, chronic gastrointestinal bleeding, epigastric mass, unexplained iron-deficiency anemia, progressive dysphagia, or persistent vomi-

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ABBREVIATIONS: *CYP2C19*, cytochrome P450 2C19; DPO, dual priming oligonucleotide; H_2RA, H_2-receptor antagonist; PM, poor metabolizer; PPI, proton pump inhibitor; V/V, variant homozygote; W/W, wild type homozygote.

ting. The participants were screened with clinical laboratory tests and interviewed with regard to their medical histories. They all were tested by upper gastrointestinal endoscopy and were treated with cimetidine for their dyspepsia. A total of 100 patients received 450 mg cimetidine daily until the dyspepsia resolved, then were tested for *CYP2C19* polymorphism.

CYP2C19 genotyping

Blood samples were collected into tubes containing 5.4 mg EDTA. Genomic DNA was extracted from leukocytes using AccuPrep® Genomic DNA Extraction kits (Bioneer Corporation, Daejeon, Korea). The *CYP2C19* polymorphism was determined using the Seeplex *CYP2C19* ACE Genotyping system (Seegene, Seoul, Korea), which is a simple, innovative dual priming oligonucleotide (DPO) primer-based multiplex polymerase chain reaction system which has high specificity and sensitivity for detecting two single nucleotide polymorphisms: the *CYP2C19*2* and *CYP2C19*3* alleles [6]. The allele specific DPO primers have an SNP in the middle of the 30-segment which maximizes disturbance of the 30-segment annealing. Multiplex PCR analysis of the genomic DNA was performed to detect alleles, together with a general primer to detect *CYP2C19* (492 bp) using 2X Mastermix (Solgent). After a preheating step at 94°C for 5 min, 35 amplification cycles were carried out in the thermal cycler under the following conditions: denaturation at 94°C for 30 s, annealing at 63°C for 30 s and extension at 72°C for 30 s. Amplification was completed with a final extension step at 72°C for 5 min.

The *CYP2C19* polymorphisms were expressed as wild type homozygote (W/W: *1/*1 allele), heterozygote (W/V: *1/*2, *1/*3 alleles), and variant homozygote (V/V: *2/*2, *2/*3, *3/*3 alleles) [7,8].

Table 1. Genetic polymorphism by *CYP2C19* genotypes of subjects

	CYP2C19 genotypes	N (%)
Wild type homozygote	*1/*1	33 (33)
Heterozygote	*1/*2	34 (34)
	*1/*3	15 (15)
Variant homozygote	*2/*2	10 (10)
	*2/*3	6 (6)
	*3/*3	2 (2)

Statistical analysis

Statistical analysis was performed using PASW Statistics 18 (IBM, Chicago, IL, USA). Continuous variables are reported as the mean and standard deviation, and categorical variables are reported as frequency and percentage. ANOVA was used to compare demographic variables and the mean durations of cimetidine treatment among the *CYP2C19* subgroups. Pearson's Chi-squared test was used to compare endoscopic diagnosis with *CYP2C19* genotypes. A two-tailed value of $p < 0.05$ was considered statistically significant.

RESULTS

Genetic polymorphism of CYP2C19 genotypes

The number of *CYP2C19* subgroups was 33 (33%) in W/W, 49 (49%) in W/V, and 18 (18%) in V/V, respectively. The frequencies of the *CYP2C19* *1/*1, *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3 genotype were 33 (33%), 34 (34%), 15 (15%), 10 (10%), 6 (6%) and 2 (2%), respectively (Table 1).

Clinical characteristics of subjects

Subject demographics, as well as clinical and laboratory findings, are shown in Table 2. There were no significant differences in the demographic, clinical and laboratory findings among the *CYP2C19* subgroups.

Endoscopic diagnosis and CYP2C19 genotypes

Chronic gastritis was the most endoscopic finding among subgroups. According to endoscopic finding, V/V was more frequency in reflux esophagitis than others. However, endoscopic diagnosis showed no significant differences among subgroups (Table 3).

The mean duration of cimetidine treatment according to CYP2C19 genotypes

The mean duration of cimetidine treatment (in weeks) was the shortest in the V/V among the *CYP2C19* genotypes

Table 2. Demographic and clinical characteristics by *CYP2C19* genotypes of subjects

	CYP2C19 genotypes			p value
	Wild type homozygote	Heterozygote	Variant homozygote	
Number of patients	33	49	18	
Age (yr)	55.2±13.5	56.1±8.7	56.3±11.8	0.909
Male (%)	10 (0.30)	21 (0.43)	7 (0.39)	0.523
BMI (kg/m^2)	22.5±2.1	22.2±1.6	22.6±1.2	0.527
Total cholesterol (mg/dl)	181.8±30.2	183.8±32.5	198.1±56.2	0.419
HDL-cholesterol (mg/dl)	52.3±7.4	57.3±11.8	54.3±9.6	0.363
LDL-cholesterol (mg/dl)	109.6±23.8	121.6±20.4	119.6±25.3	0.774
Triglyceride (mg/dl)	127.1±36.4	134.1±31.5	121.1±34.7	0.587

BMI, body mass index.
Continuous data are shown as mean±SD. Categorical data are shown as number (%).
$p < 0.05$ by ANOVA.

Table 3. Endoscopic diagnosis by *CYP2C19* genotypes of subjects

	CYP2C19 genotypes			p value
	Wild type homozygote (%)	Heterozygote (%)	Variant homozygote (%)	
Number of patients	33	49	18	0.848
Reflux esophagitis	3 (0.09)	6 (0.12)	3 (0.17)	
Chronic gastritis	26 (0.79)	34 (0.69)	12 (0.67)	
Chronic atrophic gastritis	4 (0.12)	9 (0.18)	3 (0.17)	

$p < 0.05$ by Pearson's Chi-squared test.

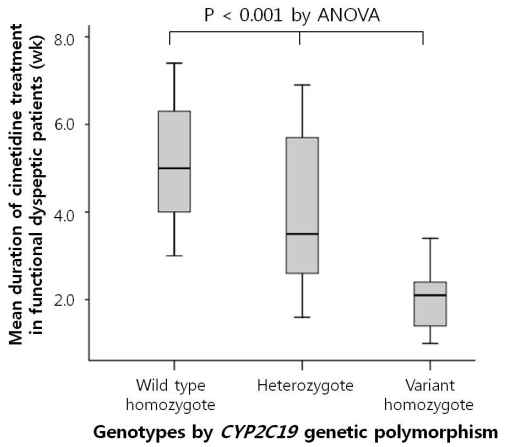

Fig. 1. Mean treatment duration (weeks) by *CYP2C19* genotypes in functional dyspeptic patients who were treated with cimetidine in Korea. It showed significant differences among the different *CYP2C19* subgroups. It was the shortest in the V/V among the *CYP2C19* genotypes (wild type homozygote: 5.1±1.5, heterozygote: 4.0±1.7, variant homozygote: 2.1±0.7; $p < 0.001$).

(W/W: 5.1±1.5, W/V: 4.0±1.7, V/V: 2.1±0.7; $p < 0.001$) (Fig. 1). There was no side effect due to cimetidine treatment.

DISCUSSION

Poor *CYP2C19*-dependent cimetidine metabolism is associated with variant homozygote genotype [8]. The frequency of variant homozygote *CYP2C19* in functional dyspeptic patients who were treated with cimetidine was 18% in this study. It was similar to that of Japanese populations (18～23%) [9]. However, it was higher than other ethnic groups; whites Americans (5.0%), African Americans (5.4%) and Mexican Americans (9.8%) [10]. It suggests that the ethnic variation acts on the *CYP2C19* polymorphism. We did not analyze *CYP2C19*17*, which is known to be an ultra-metabolizer phenotype. The frequency of *CYP2C19*17* has been reported to be very low in Asian populations [11].

There were no differences in the demographic and endoscopic features among patients with different *CYP2C19* genotypes. It was similar with the result of Sheu et al. [7]. In addition, they showed that the 1-year cumulative failure rate of on-demand therapy was significantly higher in W/W than other subgroups. However, we did not check the degree and the effect of reflux esophagitis. The variant homozygote *CYP2C19* group exhibited a significantly lower mean duration of cimetidine treatment when compared with the wild type homozygote group in this study. Although we did not prove that *CYP2C19* polymorphism is linked to dyspepsia recurrence, we showed a statistically significant association between *CYP2C19* polymorphism and the mean duration of cimetidine treatment in the subjects with functional dyspepsia.

We excluded current smokers in this study because smoking could induce dyspeptic symptoms. A previous study reported the effects of smoking on *CYP2C19* genotype in influencing *H. pylori* eradication success, although they did not examine the interaction between *CYP2C19* polymorphisms and smoking [12]. Smoking is an important factor that decreases blood flow in the stomach and increases gastric reflux into the esophagus [12].

We suggest that the interaction between smoking or recurrence of dyspepsia and *CYP2C19* polymorphisms during the cimetidine treatment be examined in the future.

A comprehensive pharmacogenomic test with genotyping of multiple genetic variations in various portions of the *CYP2C19* gene would likely provide a better prediction of the combined effects of genetic mutations on drug metabolism, which could better guide the selection of appropriate treatment durations. This study can also act as a basis for further investigation to identify the underlying genetic, epigenetic, or environmental factors in *CYP2C19* enzyme activity.

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