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Association of gastric cancer with cytochrome P450 2C19 single-nucleotide polymorphisms in Koreans

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SUMMARY

Cytochrome P450 2C19 (CYP2C19) is a clinically important enzyme involved in the metabolism of therapeutic drugs, including (S)-mephenytoin, omeprazole, proguanil, and diazepam. Individuals are characterized as either extensive metabolizers (EM) or poor metabolizers (PM) on the basis of CYP2C19 enzyme activity. The PM phenotype occurs in 2 - 5% of Caucasians, but in 18 - 23% of Asians. To clarify the association between *CYP2C19* polymorphisms and gastric cancer in Koreans, we investigated CYP2C19 genotypes (CYP2C19*1, *2, and *3) in 109 patients with gastric cancer and 211 controls. Normal (CYP2C19*1) and defective alleles were detected with polymerase chain reaction/restriction enzyme analysis. CYP2C19 has three hereditary genotypes: homozygous EM, with high enzymatic activity; heterozygous EM, with moderate enzymatic activity; and PM, with no enzyme activity. We found that CYP2C19 heterozygous EM is more closely associated with gastric cancer than is homozygous EM. Because the CYP2C19 genotype varies in Koreans, a genotyping test is desirable to prevent gastropathy recurrence in patients before their doses of omeprazole are reduced during maintenance therapy.

Key words: CYP2C19; Korean; Gastric cancer; Genotypes; SNP

INTRODUCTION

Gastric cancer is one of the most common cancers in the world and is the leading cause of death in Korea. Cytochrome P450 2C19 (CYP2C19) is a clinically important enzyme involved in the metabolism of drugs such as (S)-mephenytoin, omeprazole, proguanil, and diazepam (Xie *et al.,* 2001). CYP2C19 also plays a crucial role in either the detoxification or inactivation of potential carcinogens, and in the bioactivation of some environmental procarcinogens to reactive DNA-binding metabolites, such as nitrosamine (Kamataki *et al.,* 2002; Xing *et al.,* 2003; Agundez *et al.,* 2004). Therefore, CYP2C19 polymorphisms are considered one of the factors associated with interindividual differences in susceptibility to certain forms of cancer, as is true for other CYP enzymes (Tsuneoka *et al.,* 1996; Yokose *et al.,* 1998; Gao *et al.,* 2002; Shi *et al.,* 2004; Suzuki *et al.,* 2004; Sugimoto *et al.,* 2005). Several recent reports have related CYP2C19 polymorphisms and susceptibility to various cancers, including lung cancer (Tsuneoka *et al.,* 1996; Shi *et al.,* 2004), hepatocellular carcinoma (Chau *et al.,* 2000), esophageal cancer, and gastric cancer (Shi *et al.,* 2004)

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There are also interethnic differences in the frequencies of CYP2C19 genotypes. In eastern Asian populations, such as the Japanese, Chinese, and Koreans, the frequencies of homozygous extensive metabolizers (EMs), heterozygous EMs, and poor metabolizers (PMs) are approximately 30 - 40%, 4 - 50%, and 14 - 22%, respectively, whereas in American and European Caucasians, they are approximately 70 - 75%, 20 - 25%, and 2 - 5%, respectively (Ishizaki *et al.,* 1994; Xiao *et al.,* 1997; Xie *et al.,* 1999).

If CYP2C19 activity is a risk factor for developing gastric cancer, the higher frequency of the CYP2C19 PM genotypes in the Korean population might explain their higher incidence of gastric cancer compared with that of Caucasians. Therefore, the aim of this study was to evaluate the relationship between CYP2C19 polymorphisms and gastric cancer using CYP2C19 genotyping in Korean gastric cancer patients and healthy volunteers.

METHODS

Subjects

Gastric cancer patients (n = 109) underwent surgical or endoscopic mucosal resection between August 2003 and February 2005, at Wonkwang University Hospital, Iksan, Chonbuk, Republic of Korea. and the diagnosis of gastric adenocarcinoma was confirmed pathologically. They had a mean age (±S.D.) of 60.1 ± 12.0 years. The control group consisted of 211 healthy volunteers, who were deemed to be free of malignancy by their physicians. They had a mean age of 25.5 ± 5.4 years. All patients and control subjects gave their written informed consent prior to their enrolment in this study. The Institutional Review Boards of Wonkwang University Hospital approved this study.

Blood sampling and DNA extraction

Blood samples (3 ml collected by vein puncture into EDTA) were obtained from all subjects. Immediately after its collection, the whole blood was stored at -20°C until use. The inorganic procedure for DNA extraction was based on that of Miller *et al.* (1988). The concentration of the DNA was estimated by absorbance at 260 nm.

Primer design

We designed specific primers for exon 5 (CYP2C19*2) and exon 4 (CYP2C19*3) according to the base sequence of the CYP2C19 gene at intron 5–intron 6 and intron 4-intron 5, respectively, as previously described by de Morais *et al.* (1994), with minor modifications. For exon 5 (CYP2C19*2), the forward primer was 5′-AATTACAACCAGAGCTTGGC-3′ and the reverse primer was 5′-TATCACTTTCCAT-AAAAGCAA-3′; for exon 4 (CYP2C19*3), the forward primer was 5′-TATTATTATCTGTTAACTAATATGA-3′ and the reverse primer was 5-ACTTCAGGGCTTGG-TCAATA-3′.

Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis

To detect the CYP2C19*2 and CYP2C19*3 mutant alleles, genomic DNA (200 ng) was amplified in a PCR reaction that contained 10 × buffer, 200 μmol of dNTPs (dATP, dCTP, dGTP, and dTTP), 1.5 mM $MgCl_2$, 0.5 mmol of PCR primers, and 2.5 units of Ampli*Taq* DNA polymerase. The amplification conditions were 35 cycles of denaturation at 94°C for 30 s, annealing (at 55°C for exon 5 and 49.7°C for exon 4) for 30 s and extension at 72°C for 45 s. An initial denaturation step at 94°C for 5 min and a final extension step at 72°C for 5 min were also included. The PCR products of CYP2C19*2 and CYP2C19*3 were digested with *Sma*I and *Bam*HI enzymes, respectively. Restriction enzyme cleavage was conducted (for CYP2C19*2 at 25°C; for CYP2C19*3 at 37°C) for 8 h after the addition of 10 units of *Sma*I and *Bam*HI. The digested PCR products were analyzed on 3% agarose gels, after staining with ethidium bromide.

Data analysis

Differences in the CYP2C19 genotype frequencies

in the control and gastric cancer groups were determined using the χ^2 test. The effects of the CYP2C19 genotypes on the risk of gastric cancer development were expressed as odds ratios (OR) adjusted for age and sex, with 95% confidence intervals (CI). All *P* values were two sided, and *P* values of < 0.05 were considered statistically significant (SPSS 10.0; SPSS, Chicago, IL, USA).

CYP2C19 genotyping

Restriction analysis of the wild type appeared as two bands of digestion products (of 120 and 49 bp for CYP2C19*2, and 233 and 96 bp for CYP2C19*3). Conversely, the homozygous mutated type appeared as a single band of undigested product (169 bp for CYP2C19*2, and 329 bp for CYP2C19*3). If all products (three bands) appeared on the gel, the subject was heterozygous.

CYP2C19 genotypes were classified into three groups: the homozygous EM (*1/*1), heterozygous EM (*1/*2 or *1/*3), and PM (*2/*2, *3/*3, or *2/*3).

RESULTS

In the wild-type gene, cleavage of the PCR products yielded fragments of 120 and 49 bp for CYP2C19*2, and 233 and 96 bp for CYP2C19*3. With individuals homozygous for CYP2C19*2, the *Sma*I site in exon 5 is destroyed and the 169-bp fragment is not cut, whereas in CYP2C19*3, the *Bam*HI site in exon 4 is destroyed and the 329-bp fragment is not cut. With heterozygous individuals, all three bands (49, 120, and 169 bp for CYP2C19*2, and 96, 233, and 329 bp for CYP2C19*3) are evident. PCR products from

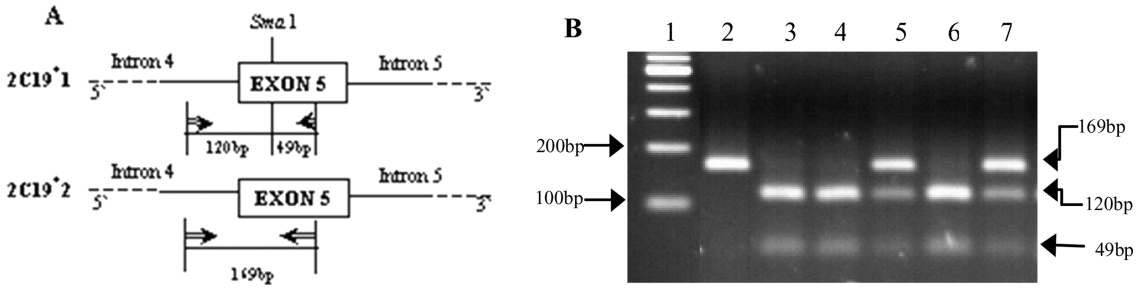

Fig. 1. PCR-based diagnostic test for CYP2C19*2 mutation. A, Strategy used to genotype genomic DNA from human blood, using PCR amplification of exon 5 followed by *Sma*I digestion (CYP2C19*2). B, Gel electrophoresis photograph showing the analysis of CYP2C19*2. Lane 1 is a 100-bp size marker. Lane 2 is homozygous (CYP2C19*2/*2), lanes 3, 4, and 6 are wild type (CYP2C19*1/*1), and lanes 5 and 7 are heterozygous (CYP2C19*1/*2).

Table 1. Frequencies of CYP2C19 genotypes in controls and patients with gastric cancer

CYP2C19 genotype	Gastric cancer no. (%)	Control no. (%)	P
Homozygous EM			
*1/*1, *1/*1	36 (33.0)	92 (43.6)	
Heterozygous EM			
*1/*2, *1/*1	44 (40.4)	61 (28.9)	
*1/*1, *1/*3	13 (11.9)	24 (11.4)	0.109
Total	57 (52.3)	85 (40.3)	
PM			
*1/*2, *1/*3	7 (6.4)	12 (5.7)	
*2/*2, *1/*1	9 (8.3)	20 (9.5)	
*1/*1, *3/*3	0 (0.0)	2 (0.9)	
Total	16 (14.7)	34 (16.1)	

The data were analyzed with the χ^2 test.

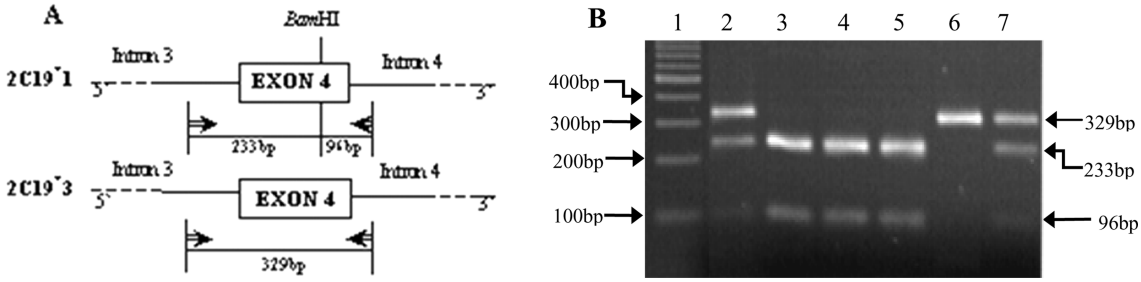

Fig. 2. PCR-based diagnostic test for the CYP2C19*3 mutation. A, Strategy used to genotype genomic DNA from human blood, using PCR amplification of exon 4 followed by *Bam*HI digestion (CYP2C19*3). B, Gel electrophoresis photograph showing the analysis of CYP2C19*3. Lane 1 is a 100-bp size marker. Lanes 2 and 7 are heterozygous (CYP2C19*1/*3), lanes 3, 4, and 5 are wild type (CYP2C19*1/*1), and lane 6 is homozygous (CYP2C19*3/*3).

Table 2. Frequencies of CYP2C19 homozygous EM and heterozygous EM genotypes in controls and patients with gastric cancer

CYP2C19 genotype	Gastric cancer no. (%)	Control no. (%)	P	OR (95% CI)
Homozygous EM *1/*1, *1/*1	36 (38.7)	92 (52.0)	0.038	0.584 (0.35 - 0.97)
Heterozygous EM *1/*2, *1/*1 *1/*1, *1/*3	57 (61.3)	85 (48.0)		

The data were analyzed with the χ^2 test.

DNA individuals with the wild-type allele(s) were cleaved by the restriction enzyme, whereas those from homozygous individuals with the mutations *2/*2 or *3/*3 lacked the *Sma*I or *Bam*HI site, respectively, and showed a single band (Figs. 1 and 2).

The frequencies of the homozygous EM, heterozygous EM, and PM CYP2C19 genotypes were 43.6%, 40.3%, and 16.1%, respectively, in the control group and 33.0%, 52.3%, and 14.7%, respectively, in the gastric cancer group. Although there was no significant difference between the total PM frequencies of the gastric cancer group and the control group, the frequency of the homozygous EM genotype was 52.0% in the total control group and only 38.7% in the gastric cancer group. However, the frequency of the heterozygous EM genotype was 48.0% in the total control group and considerably higher in the gastric cancer group, at 61.3% (OR, 0.584; 95% CI, 0.35 - 0.97).

DISCUSSION

Cytochrome P450s are the main drug-metabolizing enzymes in the human body, and always participate in the metabolism of carcinogens or procarcinogens. Some cytochrome P450s are involved in the activation of procarcinogens and some may take part in the inactivation of carcinogens. Several studies of CYP2C19 polymorphisms and their association with carcinogenesis have shown contradictory results (Wadelius *et al.,* 1999; Roddam *et al.,* 2000; Sachse *et al.,* 2002). In one example, there was no relationship between prostate cancer and CYP2C19 polymorphisms. However, Klose *et al.* (1999) have reported that CYP2C19 is only expressed in the liver and duodenum. How it functions differently in different organs has not yet been clarified. This implies that different types of cancers may have different oncological mechanisms.

Among the polymorphisms of CYP2C19, the mutations CYP2C19*2 and CYP2C19*3 are very important. In particular, the CYP2C19*3 allele is not observed in Caucasians, reflecting interethnic differences. In this study, CYP2C19*2 and CYP2C19*3 were detected in Koreans. However, the polymorphic distribution of the CYP2C19 gene does not differ in Korean gastric cancer patients and healthy Korean subjects.

However, CYP2C19 can be classified by enzyme activity and genotype into three hereditary phenotypes: homozygous EM, with higher enzymatic activity; heterozygous EM, with moderate enzymatic activity; and PM, with no enzymatic activity.

We found that the incidence of PMs in Koreans according to genotype is up to 16%, higher than that of Caucasians, which is reported to be 2% - 5% (Xiao *et al.,* 1997; Xie *et al.,* 1999). Furthermore, in this study, the heterozygous CYP2C19 EM genotype showed a higher frequency in gastric cancer patients than did the homozygous CYP2C19 EM genotype.

We can infer that PMs, caused by mutation of the CYP2C19 gene in gastric cancer patients, is associated with a higher incidence of gastric cancer in Koreans. This should be verified by data drawn from more samples. The contribution of CYP2C19 polymorphisms to the mechanism of gastric cancer in patients remains to be determined. The clinical usefulness of CYP2C19 genotyping in the prevention and screening of gastric cancer must also be investigated further.

This finding could be important for the clinical efficacy and toxicity of many therapeutic agents that are metabolized by CYP2C19, such as omeprazole. Thus, phenotype-genotype correlations, using a healthy population as the reference standard, may be a means by which to monitor changes in drug-metabolizing enzyme activities in diseases such as cancer.

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