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Research Article

CYP2C19 Polymorphisms in Patients with Gastric and Colorectal Carcinoma

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Abstract

Background: It has been reported that up to 80% of human cancers arise as a consequence of environmental exposure and host susceptibility factors. Environmental carcinogens are predominantly metabolized by the cytochrome P450 (CYP) superfamily of drug- or xenobiotic-metabolizing enzymes. Genetic variations in these enzymes affect individuals' susceptibility to carcinogens.

Aim of the study: The aim of this study was to evaluate the relationship between CYP2C19 polymorphism and susceptibility to these cancers by means of CYP2C19 genotyping among Turkish subjects.

Methods: DNA of subjects were isolated from leukocytes by high pure template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany) and genotypes were detected by LightCycler *CYP2C19* Mutation Detection Kit by real-time PCR with LightCycler instrument (Roche Diagnostics, cat. no. 3113914).

Results: Being male was associated with a 3.5-fold (OR: 4.27, CI: 2.27–8.05) and 4.27-fold (OR: 3.50, CI: 1.948–6.301) risk for colorectal and gastric carcinoma, respectively. The *CYP2C19*3* heterozygote genotype was not found in either gastric or colorectal carcinoma patients. Although the frequency of CYP2C19*2 heterozygote genotype is high in patients with gastric and colorectal carcinoma, it is not significantly associated with cancer (OR: 1.79, CI: 0.829–3.865 and OR: 1.998, CI: 0.961–4.154, respectively).

Conclusion: Although the frequency of *CYP2C19*2* heterozygote genotype is high in our patients with gastric and colorectal carcinoma, there is no the relationship between CYP2C19 polymorphism and susceptibility to these cancer.

Key Words: CYP2C19; polymorphism; gastric and colorectal carcinomas.

Introduction

Exogenous or endogenous chemicals in their native or metabolized forms are able to affect DNA integrity and lead to cancer if the exposure persists. It has been reported that up to 80% of human cancers arise as a consequence of environmental exposure and host susceptibility factors *(1)*. The environmental procarcinogen hypothesis states that exogenous or endogenous chemicals are procarcinogens that can be metabolically activated to form highly reactive proximate carcinogens. The formation of these reactive agents is catalyzed predominately by the cytochrome P450 (CYP) superfamily of drug- or xenobiotic-metabolizing enzymes. The resulting

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metabolites can interact directly with DNA and lead to tumor initiation and/or promotion *(2–4)*. Therefore, polymorphisms of the genes encoding for this enzyme involved in the metabolism of these hazardous compounds may be related to interindividual differences in cancer susceptibility *(1)*

Individuals vary widely in their susceptibility to carcinogens. One attractive genetic mechanism to account for this variability is the activity of polymorphically expressed cytochrome P450 enzymes that activate procarcinogens or conversely detoxify carcinogens *(5)*. Cytochrome P450 2C19 (CYP2C19) is a clinically important metabolic enzyme that plays a critical role in the oxidative metabolism of a variety of endogenous and exogenous compounds, including a number of therapeutic drugs, such as S-mephenytoin, omeprazole, diazepam, proguanil, propranolol, and certain antidepressants *(2,5)*.

Normally, Asians had a higher incidence of CYP2C19 poor metabolizers, which was usually about 13–16%, but in Caucasians it was only 1–3% *(6)*. Phenotyping analyses revealed an association between CYP enzyme activity and the risk of developing several forms of cancer *(7)*. Research carried out in the last decade demonstrated that several CYP enzymes are polymorphic due to single nucleotide polymorphisms, gene duplications, and deletions. As genotyping and phenotyping procedures became available for most human CYP, an impressive number of association studies on CYP polymorphisms and cancer risk were conducted *(7)*.

Worldwide in 1996, an estimated 876.000 new cases of colorectal cancer occurred—445,000 in males and 431,000 in females. Less than one-third of colorectal cancer cases occur in developing countries. In developing countries, colorectal cancer is the second most common cancer in both sexes *(8)*. Although gastric cancer is one of the most common malignancies worldwide, the pathogenesis of this disease and the molecular genetic events that contribute to its development are poorly understood. The diverse and changing pattern of worldwide gastric cancer incidence rates indicates a complex role for environmental risk factors in this disease *(9,10)*.

As susceptibility to colorectal and gastric cancer, two of the most common forms of the cancer in the world have been associated with several environmental and dietary risks. The aim of this study was to evaluate the relationship between CYP2C19 polymorphism and susceptibility to these cancer by means of CYP2C19 genotyping among Turkish subjects.

Material and Methods

Subjects

The study subjects comprised 105 patients with colorectal carcinoma and 77 gastric cancers attending the Clinic of the General Surgery Department, University of Mersin and the Clinic of the General Surgery Department, University of Kocaeli, and 105 healthy controls who visited our hospital for an annual check up. Control subjects were selected among healthy persons without a history of malignancy, thyroid function disorders, hypertension, diabetes mellitus, liver and renal dysfunction, anemia, osteoporosis, and inflammatory and autoimmune disease. The study was carried out according to the declaration of Helsinki and approved by Mersin University, Faculty of Medicine, and Investigational Review Board. Informed consent was obtained from all participating patients. All patients with colorectal and gastric carcinoma had undergone surgical procedures depending on the location of their tumors. All of the specimens examined had been diagnosed histopatologically.

DNA Extraction and Genotyping of CYPC19

Blood was collected in EDTA-containing tubes and DNA was extracted from the leukocytes by high pure template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany). *CYP2C19*2, CYP2C19*3* alleles were detected by using LightCycler *CYP2C19* Mutation Detection Kit by real-time PCR with LightCycler instrument (Roche Diagnostics, cat. no. 3113914).

Statistical Analysis

Odds ratios and their confidence intervals were calculated using binary logistic regression analysis. We used SPSS 10.5 for this analysis and for related count and percents.

Results

We determined *CYP2C19*2* and *CYP2C19*3* alleles in patients with colorectal and gastric carcinoma. There was no significant association age between cases and control: 60%, 50%, and 31% of

Table 1
CYP2C19 and *CYP2C19* Alleles and Their Related Odds Ratios for Colorectal and Gastric Carcinoma

Disease	Variables	Patients *n* (%)	Controls (*n*=105) *n* (%)	Odds ratio	Confidence interval	*p* value
Colorectal carcinoma	*CYP2C19*2 wild*	81 (77.1)	88 (83.8)	Reference	—	—
n = 105	Heterozygote	24 (22.9)	17 (16.2)	1.998	0.961–4.154	0.064
	*CYP2C19*3 wild*	105 (100)	105 (100)	Reference	—	—
	Heterozygote	—	—	—	—	—
	Male	60 (57.1)	31 (29.5)	3.503	1.948–6.301	0.000
	Female	45 (42.9)	74 (70.5)	0.285	0.159–0.513	1
Gastric carcinoma	*CYP2C19*2 wild*	56 (72.7)	88 (83.8)	Reference	—	—
n = 77	Heterozygote	21 (27.3)	17 (16.2)	1.790	0.829-3.865	0.138
	*CYP2C19*3 wild*	77 (100)	77 (100)	Reference	—	—
	Heterozygote	—	—	—	—	—
	Male	50 (64.9)	30 (39.0)	4.270	2.271–8.053	0.000
	Female	27 (35.1)	47 (61.0)	0.234	0.124–0.440	1

‡ ORs (odds ratio); CI (confidence interval) from logistic regression; *n*, number of sample; *p*, values of significance with difference of each group; *Odds ratio cannot be calculated; wild allele are used as reference.

colorectal and gastric carcinoma patients and controls were male, respectively.

Risk evaluations of these polymorphisms are represented in Table 1. Being male is related with 4.27-fold (OR: 4.27, CI: 2.27–8.05) and 3.5- fold (OR: 3.50, CI: 1.948–6.301) risk for gastric and colorectal carcinoma, respectively. There is no mutant genotype in both *CYP2C19* alleles in all groups. Also, *CYP2C19*3* heterozygote genotype were not detected in all subjects including patients and controls. Although the frequency of *CYP2C19*2* heterozygote genotypes are high in patients with colorectal and gastric carcinoma (OR: 1.998, CI: 0.961–4.154; OR: 1.79, CI: 0.829–3.87, respectively), there is no significant risk for these carcinomas.

Discussion

Susceptibility to cancer is mediated by genetically determined differences in the effectiveness of activation and detoxification of potential carcinogens. Thus, polymorphisms in activating and detoxifying metabolic enzymes are associated with an altered risk for certain cancers *(11)*.

Although gastric cancer and colorectal cancer are some of the most common malignancies worldwide, the pathogenesis of this disease and the molecular genetic events that contribute to its development are poorly understood *(8,9)*. Genetic polymorphisms of drug-metabolizing enzymes such as arylamine N-acetyltransferases, glutathione S-transferase, cytocrome P-450 enzymes have recently been shown to affect susceptibility to chemical carcinogenesis. However, the molecular mechanisms of individual susceptibility to these cancers have not been fully understood *(12)*.

Cytochrome P450s are the main drug-metabolizing enzymes in thehuman body, and are always found to participate in the metabolism of carcinogens or procarcinogens. Some are involved in the activation of procarcinogens, some may take part in the inactivation of carcinogens. That depends on what kind of carcinogens and what kind of cancers, and what type of mechanism of carcinogenesis *(6)*.

CYP2C19—one of the most important cytochrome P450s—is known as a key enzyme in the in vivo metabolism of a number of related hydantoins and barbiturates, as well as in the metabolism of structurally unrelated drugs such as omeprazole, lansoprazole, progunil, mephenytoin, and citalopram. Individuals can be divided into two groups, poor metabolizers (PMs) and extensive metabolizers (EMs), depending on the hydroxylation ability of S-mephenytoin *(5)*.

The molecular basis of genetic polymorphism of the CYP2C19 gene, which causes impaired drug

metabolism, has been determined in recent years and at least two genetic defects (*CYP2C19*2* and *CYP2C19*3*) in the CYP2C19 gene have been shown to be responsible for the PM phenotype involved in the oxidation of a prototype substrate S-mephenytoin in vivo. *CYP2C19*2*, which shows a single base mutation (G_{681}gA) in exon 5, is one genetic difference and it has been shown to be present in both Japanese and Caucasian populations. The other mutation, the *CYP2C19*3* type, consists of a premature stop codon (G_{636}gA) in exon 4, and is found only in Asians, including both Japanese and Chinese individuals *(13)*. An individual who inherits two mutant CYP2C19 alleles, whether the same kind (*2/*2, *3/*3) or a different kind (*2/*3), has a reduced capacity to metabolize CYP2C19 substrates and is a PM. Individuals who are homozygous (*1/*1) or heterozygous (*1/*2, *1/*3) for wild-type CYP2C19*1 have a efficient enzyme to metabolize CYP2C19 substrates and are EMs *(6)*.

Several studies on CYP2C19 polymorphism and its association with carcinogenesis have shown self-contradictory results. Wadelius et al. *(14)* found no association between CYP2C19 polymorphism and prostate cancer. Roddam et al. *(15)* reported an increased risk of CYP2C19 PMs to develop adult acute leukemia and Sachse et al. *(16)* found CYP2C19*2 had an decreased risk of colorectal cancer *(6)*. CYP2C19 PMs have a high incidence of esophagus cancer, stomach cancer, and lung cancer; conversely, they have a low incidence of bladder cancer. Chau et al. *(17)* reported the PM phenotype caused by the mutation of CYP2C19 gene in cirrhotic patients with HCV infection is associated with a high risk for developing hepatocellular carcinoma. Modugno et al. *(2)* concluded that CYPs involved in estrogen metabolism are expressed in both tumor and nontumor breast tissue. Local activation of estrogen to potentially reactive metabolites by the CYPs in breast tissue may play a role in initiating and promoting the carcinogenic process.

Although the frequency of *CYP2C19*2* heterozygote genotype is high in patients with colorectal and colorectal carcinoma (OR: 1.998, CI: 0.961–4.154; OR: 1.79, CI: 0.829–3.865, respectively), there is no significant risk for these carcinomas.

If unequivocal biomarkers of genetic susceptibility to cancer and toxicity can be developed successfully, then identification of individuals at increased risk would be very helpful in the fields of public health and preventive medicine.

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