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Cytochrome p450 2E1 polymorphisms and the risk of gastric cardia cancer

Lin Cai, Zong-Li Zheng, Zuo-Feng Zhang

Lin Cai, Zong-Li Zheng, Department of Epidemiology, Fujian Medical University, Fuzhou 350004, Fujian Province, China
Zuo-Feng Zhang, Department of Epidemiology, UCLA School of Public Health, Los Angeles, CA, USA

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Correspondence to: Lin Cai, M.D., Ph.D., Department of Epidemiology, UCLA School of Public Health, 71-225 CHS, Box 951772, 10833 Le Conte Avenue, Los Angeles, CA 90095-1772, USA. lcain@ucla.edu

Telephone: +1-310- 825-8418

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development of GCC, and that the risk increases significantly in smokers.

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Abstract

AIM: Genetic polymorphisms of drug-metabolizing enzymes have recently been shown to affect susceptibility to chemical carcinogenesis. Cytochrome P450 2E1 (*CYP2E1*) enzyme catalyzes the metabolism of many procarcinogens, such as *N*-nitrosamines and related compounds. The gene coding for this enzyme is polymorphic and thus may play a role in gastric cardia cancer (GCC) etiology. In this hospital-based case-control study, we evaluate the relationship between genetic polymorphisms of *CYP2E1* and the risk of GCC.

METHODS: The study subjects comprised 159 histologically confirmed GCC cases identified via hospital cancer registry and surgical records at five hospitals in Fuzhou, Fujian Province, China, between April and November 2001. Controls were 192 patients admitted to the same hospitals for nonmalignant conditions. The genotypes of *CYP2E1* were detected by a PCR-based RFLP assay. The odds ratios were estimated by logistic regression analyses and were adjusted for potential confounding factors.

RESULTS: The distribution of three genotypes of *CYP2E1* in GCC cases and controls was significantly different ($\chi^2 = 16.04$, $P < 0.01$). The frequency of the *CYP2E1* (*c1/c1*) genotype in GCC cases and controls was 60.4% and 40.1%, respectively. The *CYP2E1* (*c1/c1*) genotype was associated with an increased risk for GCC (the adjusted (OR) was 2.37, 95% confidence interval (CI): 1.52-3.70). Subjects who carried the *CYP2E1* (*c1/c1*) genotype and were habitual smokers were at a significantly higher risk of developing GCC (OR = 4.68, 95%CI: 2.19-10.04) compared with those who had the *CYP2E1* (*c1/c2* or *c2/c2*) genotype and did not smoke.

CONCLUSION: These results suggest that the *CYP2E1* genotype may influence individual susceptibility to

INTRODUCTION

Gastric cardia cancer (GCC) has shown a rapid increase in incidence in many developed countries in the last 20 years [1-5]. Despite a steady fall in incidence of non-cardia cancer, gastric cancer remains a significant cause of mortality and morbidity worldwide [6,7]. Epidemiological studies have reported that exposure to *N*-nitrosamines and related compounds may be important factors for gastric carcinogenesis [8-11]. Environmental chemical carcinogens require metabolic activation by host enzymes [12-15]. *CYP2E1* is of critical importance in the metabolic activation of many low-molecular-weight carcinogens, including *N*-nitrosamines [16]. The gene coding for this enzyme is polymorphic and thus may also be involved in this process [17,18]. Several studies described significant associations between *CYP2E1* polymorphisms and the incidences of human cancer, e.g., in esophageal cancer [19], lung cancer [20], nasopharyngeal carcinoma [21] and colorectal cancer [22-24]. In contrast, other studies showed no association [25-27]. To date there have been no studies on the relationship of this genotype to GCC risk [28]. We hypothesized that if *N*-nitroso compounds play a role in GCC, and smoking is a significant source of exposure for these compounds, then the polymorphisms may be associated with this disease and may modify the association of smoking with GCC. In the present study, we evaluated the possible relevance of Cytochrome P450 2E1 polymorphisms to GCC.

MATERIALS AND METHODS

Study subjects

The cardia cancer group ($n = 159$) consists of all inpatients who were diagnosed with primary cardia cancer and were identified between April and November of 2001 from five hospitals: First-accessory hospital of Fujian Medical University, Xiehe hospital-affiliated Fujian Medical University, Fujian

provincial hospital, Tumor hospital of Fujian province and Zong hospital of Fuzhou. Frequency matched controls ($n = 192$) were continuously recruited by gender and age (± 3 years) during the same period in the same hospitals, and were confirmed to be cancer-free and with no history of cancer. All subjects were ethnic Han Chinese and residents of Fuzhou, Fujian province, China.

Interview

In-person interviews were conducted at the hospitals by trained interviewers. A structured questionnaire was composed of items such as general characteristics, personal medical history, family cancer history, smoking and drinking history, and dietary factors. Blood samples were collected from each participant for genotyping.

Genotyping

DNA was extracted from the coagulated blood using DNAzol reagent according to the manufacturer's instructions. Genotyping for polymorphisms of *CYP2E1* (GenBank ID: NM_000773) was detected using the PCR-RFLP technique. PCR products were generated by using 100 ng of genomic DNA in 25 μ L volume reactions containing 10 mmol/L Tris-HCL (pH 8.3), 50 mmol/L KCL, 2.0 mmol/L MgCl₂, 0.2 mmol/L each dNTP, 0.25 μ mol/L each outer oligonucleotide primer (forward, 5'-CCAGTCGAGTCT-ACATTGTCA-3'; reverse, 5'-AGACCTCCACATT-GACTAGC-3') and 1.5 U *Taq* DNA polymerase. The PCR amplification consisted of an initial 5-min incubation at 94 °C, followed by 35 cycles of denaturing at 94 °C for 30 s and annealing at 58 °C for 30 s, with an extension at 72 °C for 1 min. The reaction was terminated after a final extension of 10 min at 72 °C. The PCR-amplified DNA fragments including the polymorphic site were digested with the restriction enzymes *Pst*I at 37 °C overnight, and subjected to electrophoresis on 2.0% agarose gel containing 0.5 μ g/mL ethidium bromide for visualization under UV light^[29]. A combination of 435- and 118-bp fragments represented *c2/c2* genotype; a combination of 553-, 435- and 118-bp fragments represented *c1/c2*; only a 553-bp fragment represented *c1/c1*. To ensure quality control, genotyping was performed with blinding to case-control status.

Table 1 Select characteristics of cardia cancer cases ($n = 159$) and controls ($n = 192$)

Variable	Cases		Controls		P
	n	(%)	n	(%)	
Age (yr)					
≤59	62	39.0	93	48.4	
60-69	61	38.4	60	31.3	
≥70	36	22.6	39	20.3	0.196
Sex					
Female	30	18.9	52	27.1	
Male	129	81.1	140	72.9	0.070
Education (yr)					
<12	95	59.7	89	46.4	
≥12	64	40.3	103	53.6	0.012

Statistical analysis

χ^2 were used to evaluate case-control differences in the distribution of genotypes. Unconditional logistic regression was used to estimate the odds ratios and 95%CI, and were adjusted for potential confounding factors.

RESULTS

The relevant characteristics of the study subjects are shown in Table 1. Eighty-one percent of cases and 72.9% of controls were male. The distribution of age and gender among cases and controls were not statistically different. GCC cases (59.7%) had a higher proportion of low educational level than controls (46.4%). Detailed results for GCC risk factors in this study have been published elsewhere.

The overall genotype frequencies for *c1/c2*, *c2/c2*, and *c1/c1* of *CYP2E1* were 47.58% (157/351), 3.13% (11/351), and 49.29% (173/351), respectively. The frequency of the C1 and C2 alleles in the control group was 68% and 31%, respectively. As shown in Table 2, 60.4% ($n = 96$) of cases and 40.1% ($n = 77$) of controls had the *c1/c1* genotype. The frequency of the *CYP2E1 c1/c1* genotype was significantly different between the GCC cases and the controls ($\chi^2 = 16.04$, $P < 0.01$), and the risk for the *c1/c1* homozygous subjects to have GCC is three-fold times greater than it is for the *c2/c2* homozygous and *c1/c2* heterozygous subjects (OR = 2.37, 95%CI: 1.52-3.70).

Of all subjects, 57.2% (91/159) of cases and 43.8% (84/192) of controls were smokers (OR = 1.58, 95%CI: 0.96-2.61). To further explore the relationship of smoking, *CYP2E1* polymorphisms and GCC, we performed an analysis of association between smoking and the *CYP2E1* genotype, separately in cases and controls. Table 3 presents the joint effects on GCC. The proportion of smokers with the *CYP2E1 c1/c1* genotype was significantly higher in patients with GCC (61/159, 38.36%) than in the controls (24/192, 12.50%). The greatly elevated risk associated with smoking was observed in the *CYP2E1 c1/c1* genotype group (taking non-smokers with the *c1/c2* or *c2/c2* genotype as the reference group). Cigarette smoking in subjects with the *c1/c1* genotype was associated with a four-fold higher risk (OR = 3.94, 95%CI: 1.60-9.67) compared to their non-smoking counterparts. There was an increasing trend in ORs with the number of cigarettes smoked per day (OR: 2.59 for <20 cigarettes a day; OR: 4.17 for ≥20 cigarettes a day). The strongest association with the GCC risk (OR = 8.44, 95%CI: 2.58-27.59) was observed among individuals carrying the *CYP2E1 c1/c1* genotype and having a long history of smoking (≥30 years), even after adjustment for potential confounding factors.

Table 2 Association of *CYP2E1* polymorphisms with cardia cancer risk

CYP2E1	Cases		Controls		OR (95%CI) ¹
	N	%	N	%	
<i>C1/c2</i>	57	35.8	110	57.3	1.0
<i>C2/c2</i>	6	3.8	5	2.6	
<i>C1/c1</i>	96	60.4	77	40.1	2.37 (1.52-3.70)

¹Adjusted for age (continuous), gender and educational level. $\chi^2 = 16.04$, $P < 0.01$.

Table 3 Associations of cardia cancer risk with joint distribution of *CYP2E1* genotypes and smoking status

Smoking status	<i>CYP2E1</i>	Cases		Controls		OR (95%CI) ¹	OR (95%CI) ²
		<i>n</i>	%	<i>n</i>	%		
Smoking							
Never	<i>c1/c2</i> or <i>c2/c2</i>	33	20.75	55	28.65	1	1
Ever	<i>C1/c1</i>	35	22.01	53	27.60	1.05 (0.56–1.99)	1.03 (0.52–2.06)
Ever	<i>c1/c2</i> or <i>c2/c2</i>	30	18.87	60	31.25	0.85 (0.40–1.78)	0.91 (0.39–2.14)
Ever	<i>C1/c1</i>	61	38.36	24	12.50	4.68 (2.19–10.04)	3.94 (1.60–9.67)
Daily consumed cigarettes							
1–19	<i>c1/c2</i> or <i>c2/c2</i>	8	5.03	15	7.81	0.81 (0.27–2.39)	0.62 (0.18–2.16)
1–19	<i>C1/c1</i>	9	5.66	6	3.13	2.99 (0.88–10.19)	2.59 (0.54–12.45)
≥20	<i>c1/c2</i> or <i>c2/c2</i>	22	13.84	45	23.44	0.90 (0.41–1.98)	1.04 (0.41–2.67)
≥20	<i>C1/c1</i>	52	32.70	18	9.38	5.34 (2.38–11.95)	4.17 (1.63–10.63)
Smoking duration (yr)							
1–29	<i>c1/c2</i> or <i>c2/c2</i>	10	6.29	25	13.02	0.71 (0.27–1.87)	0.82 (0.27–2.49)
1–29	<i>C1/c1</i>	19	11.95	18	9.38	2.10 (0.85–5.21)	1.99 (0.61–6.49)
≥30	<i>c1/c2</i> or <i>c2/c2</i>	20	12.58	35	18.23	1.10 (0.47–2.57)	1.00 (0.37–2.69)
≥30	<i>C1/c1</i>	42	26.42	6	3.13	13.32 (4.50–39.45)	8.44 (2.58–27.59)

¹Odds ratios adjusted for age (continuous), gender and educational level. ²Odds ratios adjusted for age (continuous), gender, educational level, family cancer history, vegetables and fruit consumption, pickled and salted food consumption, alcohol consumption, and refrigerator use.

DISCUSSION

Gastric cancer is a common cancer in China as well as the rest of the world. Epidemiological studies have shown an association between nitrosamine exposure and increased risk of gastric cancer^[30]. Besides diet, exogenous exposure to nitrosamines can occur through use of tobacco products^[31,32]. Recently, several studies have found a higher relative risk of smoking for GCC^[33–35]. A new prospective study found that the relative risk of current smokers was 2.4 for cardia cancer and 1.7 for all sites^[36,37]. In our previous report, we found an elevated risk of gastric cancer with smoking habit, more distinct with the GCC than non-GCC.

Gastric carcinogenesis is a multistep process in which genetic and environmental factors interact in the development of cancer. Interindividual genetic differences in susceptibility to chemical carcinogens are among the most important host factors in human cancer^[38–40].

It has been proposed that various host factors affect susceptibility to cancer, even following the same exposure to environmental carcinogenic factors^[41–43]. *CYP2E1* is one of the main enzymes for bioactivation of tobacco-related substances, and its polymorphisms may be associated with or be risk factors for various forms of cancers^[44–46].

Our present data demonstrate that individuals carrying the *CYP2E1 c1/c1* genotype were at increased risk for GCC. Moreover, subjects who carry this genotype and have a history of heavy cigarette smoking were at markedly greater increased risk (more than four-fold) for GCC. These results suggested that the interaction of the *CYP2E1* polymorphism with smoking has a great influence on susceptibility to GCC. To our knowledge, this is the first study to examine the association of *CYP2E1* polymorphisms with GCC.

The development of GCC may be associated with *N*-nitroso compound exposures. Tobacco smoke contains hundreds of known and probable human carcinogens^[47]. Specific chemicals in tobacco smoke include polycyclic aromatic hydrocarbons (PAHs), *N*-nitrosamines, aromatic amines and others^[48,49]. A critical review summarizing data for tobacco constituents proposed that tobacco-specific

nitrosamines and PAHs are classes of compounds that mostly affect human cancer risk^[50,51]. Nitrosamines require metabolic activation by cytochrome P450 enzymes before they bind to DNA, initiating the carcinogenic process^[52]. Evidence exists that carcinogen-DNA adduct levels are affected by genetic predispositions. Studies indicate that carcinogen-DNA adducts are related to cancer risk^[53]. Smoking-related DNA adducts have been detected in human gastric cancer of smokers. *CYP2E1* is a key-activating enzyme because it catalyzes the α -hydroxylation of many nitrosamines. Its activity shows significant interindividual variation, due in part to inherited alterations of the structural gene^[54].

The frequency of the *c1/c1* genotype among controls in this study is similar to previously published estimates in Chinese populations. Tan *et al*^[55], studied 150 cases with esophageal cancer, 146 cases with esophageal dysplasia, and 150 normal controls in Linxian, China and found that the distribution of *CYP2E1 c1/c1* allele frequency was significantly different between controls (44.0%) and cases with cancer (71.3%) or cases with dysplasia (70.6%).

In conclusion, the present case-control study suggested that the individuals carrying the *CYP2E1 c1/c1* genotype have a higher risk for GCC. The *CYP2E1 c1/c1* genotype is considered as one of the possible susceptibility genes, and the risk increases significantly in smokers. Because of the limited number of subjects, this study does not have appropriate power to detect the interaction between smoking and other factors. Further studies are required to clarify the relationship between *CYP2E1* and GCC susceptibility.

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