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E-cadherin gene C-160A promoter polymorphism and risk of non-cardia gastric cancer in a Chinese population

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Abstract

AIM: To test the hypothesis that *E-cadherin* gene (CDH1) C-160A promoter variant genotype is associated with an increased risk for developing gastric cancer.

METHODS: In this population-based case-control study of gastric cancer in Jiangsu Province, China, we performed polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to genotype the C-160A polymorphism of CDH1 promoter in 206 non-cardia gastric cancer patients and 261 age- and sex-matched but unrelated cancer-free controls.

RESULTS: The frequencies of genotypes CC, CA and AA were 57.8%, 36.4% and 5.8% in gasfric cancer cases, respectively, and 58.2%, 34.9% and 6.9% in controls respectively. The distributions of CDH1 genotypes were not significantly different between gastric cancer cases and controls ($P = 0.87$ for genotype frequency and $P = 0.92$ for allele frequency). Compared with the CC genotype, the CA and AA genotypes were not associated with an increased risk for non-cardia gastric cancer (adjusted odds ratios (OR) = 1.15, and 95% confidence interval (95% CI) = 0.78-1.72 for CA genotype, and OR = 0.90 and 95% CI = 0.42-2.01 for AA genotype).

CONCLUSION: *E-cadherin* gene C-160A promoter polymorphism may not play a major role in the etiology of non-cardia gastric cancer in Chinese population.

Key words: Gastric cancer; *E-cadherin* gene; Promoter; Polymorphism

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INTRODUCTION

Gastric cancer is the second most frequent malignancy worldwide, accounting for 8.7% of all cancers and 10.4% of all cancer deaths[1]. Distribution of gastric cancer has geographical variations, with the highest incidence in China and Eastern Asian countries[1,2]. Currently, about 39% of gastric cancer cases occur in Chinese population, ranking the leading cause of cancer-mortality in China, particularly in rural areas[1]. To date, the etiology of gastric cancer is unclear, although multiple factors[3,4] are thought to play a role in gastric carcinogenesis, including diet[5,6], tobacco smoking and alcohol consumption[7,8], *Helicobacter pylori* (*H pylori*) infection[9,10], and genetic factors[11].

Epidemiological studies have demonstrated that genetic predisposition plays an important role in gastric cancer risk with postulated molecular mechanisms underlying such genetic susceptibility[12,13]. Support for genetic susceptibility is evidenced by the aggregation of gastric cancer in the first-degree relatives of gastric cancer patients, and these family members have the risk of developing gastric cancer 2-3 times that of the general population[3]. However, family studies are methodologically limited, because they do not distinguish genetic from environmental factors, as family members tend to have common environments and lifestyles[14].

Epithelial *E-cadherin* is a cell surface glycoprotein that is responsible for Ca^{2+}-dependent cell-cell adhesion and plays an important role in the establishment and maintenance of normal epithelial polarity and organization[15]. Loss of E-cadherin expression in humans is associated with cancers including familial gastric cancer[16]. Truncating mutations in the *E-cadherin* gene (CDH1) are the most consistent genetic alterations observed in sporadic and hereditary gastric cancer[17,18]. In addition to these inactivating mutations, a CDH1 promoter polymorphism at position -160 from the transcriptional start site was reported to lead to transcriptional downregulation of the gene *in vitro*, and the variant A-allele was shown to decrease the transcriptional efficiency by 68% compared with C-allele and therefore this promoter polymorphism has been speculated as a potential genetic marker for susceptibility to cancer[19]. Recently, in a hospital-based case-control study of gastric cancer, Wu *et al*[20] reported that individuals with *E-cadherin* -160 A/A genotype had a significantly decreased risk of gastric cancer, suggesting that A-allele may be a protective allele against gastric cancer. However, the results from different ethnic populations remain inconclusive. A recent haplotype analysis suggest that CDH1 C-160A promoter polymorphism might be in linkage disequilibrium with a distinct etiological locus or acts in combination with other functional variants in or near the CDH1 region[21]. To further test the hypothesis that the CDH1 C-160A promoter polymorphism is associated with the risk of gastric cancer, we genotyped this polymorphism in a population-based case-control study of 206 patients with incident gastric non-cardia cancer and 261 age- and sex-matched cancer-free controls.

MATERIALS AND METHODS

Subjects

This population-based case-control study was conducted in Huaian and Jintan counties, two areas of high cancer mortality, in central Jiangsu Province, China, as described previously[22]. Briefly, we ascertained 341 histologically confirmed gastric adenocarcinoma cases diagnosed between January 1, 1998, and December 31, 2000, through the cancer registry system from these two counties. All patients were local residents with informed consent to donate a blood sample. Patients with secondary and recurrent tumors were excluded. Because it was reported that there was an etiological difference between gastric cardia and non-cardia cancers[23], we only included 209 non-cardia gastric cancer cases in this study. The controls were 270 cancer-free individuals randomly selected from the neighbouring counties. All study subjects were interviewed by a trained interviewer using a pre-tested questionnaire to obtain information concerning occupational history, dietary habits, smoking and drinking status, individual and family histories of digestive diseases, including cancer. After the interview, approximately 5 mL venous blood sample was collected from each subject after the informed consent was obtained. DNA quality or quantity was insufficient in 3 cases and 9 controls for PCR; thus, the study population consisted of 206 cases and 261 controls in the final analysis. Individuals who smoked once a day for over 1 year were defined as smokers, and individuals who consumed alcohol 3 or more times a week for over 6 mo were considered drinkers.

Serologic detection of antibody IgG to H pylori

Serum was separated from the blood sample within 4 h after collection and stored at -20℃ for testing IgG antibody to *H pylori*. Serum IgG antibody to *H pylori* was measured with an enzyme-linked immunosorbent assay (anti-*H pylori* enzyme immunoassay, Jinmei Biotech, Inc., Shenzhen, China). The OD value of the serum sample >2.1 was considered positive, and the value equal to or below 2.1 was considered negative.

Genotyping

Genomic DNA was isolated from peripheral blood lymphocytes by proteinase K digestion and phenol-chloroform extraction. Polymerase chain reaction (PCR)- restriction fragment length polymorphism (RFLP) assay was used to type the CDH1 -160 C→A polymorphism. We designed two primers of 5'-TCCA GGTCTTAGTGAGAACCA-3' (sense) and 5'-CCACCCGGCCT CGCATAGAC-3' (anti-sense) which generated a 135-bp fragment. The fragment was amplified in 20 μL reaction mixture containing about 50 ng of genomic DNA, 5 pmoL of each primer, 2.5 mmol/L each dNTP, 1×PCR buffer, 1.5 mmol/L $MgCl_2$ and 2 U Taq DNA polymerase (Jinmei Biotech, Inc., Shenzhen, China). The mixtures were subjected to PCR with an MJ-PTC-200 DNA engine (MJ Research, Inc., Watertown, USA). The PCR conditions consisted of an initial melting step at 95 ℃ for 5 min followed by 35 cycles at 95 ℃ for 30 s, at 62 ℃ for 40 s and at 72 ℃ for 45 s, and a final step at 72 ℃ for 10 min. The PCR products were checked on 1.5% agarose gel, and then subjected to RFLP analysis, digested with 5 U of restriction enzyme *Hinc* II (New England Biolabs Ltd, USA) at 37 ℃ for 4 h, and the genotypes were discriminated on 3% NuSieve 3:1 agarose (FMC BioProducts, Rockland, ME) gel with ethidium bromide. The wild-type C-allele produced a single 135-bp fragment, and the polymorphic A-allele produced 2 fragments of 110-bp and 25-bp. About 10% of the samples were randomly genotyped again, and the reproducibility was 100%.

Statistical analysis

Differences in distributions of selected demographic variables, smoking, alcohol consumption, and CDH1 genotype frequencies between gastric cancer cases and controls were evaluated by the χ^2 test. The association between CDH1 promoter polymorphism and gastric cancer was estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from both univariate and multivariate logistic regression analyses after adjusted for age, sex, area, smoking, alcohol consumption, tea drinking, *H pylori* infection and family history of gastric cancer. The genotype data were further stratified by subgroups of age, sex, smoking, alcohol drinking, tea drinking, *H pylori* infection and family history of gastric cancer. All the statistical analyses were performed with the statistical analysis system software (Version 8.1; SAS Institute Inc., Cary, NC).

RESULTS

Selected characteristics of gastric cancer cases and controls are summarized in Table 1. According to the genotyping results and questionnaire data, we included 206 non-cardia gastric cancer cases and 261 cancer-free controls who had all these data available in this analysis. All the subjects were Han nationalities. The mean age was 61.31 years (range 31-84 years) for the cases and 61.25 years (range 30-87 years) for the controls. The differences in distribution of age and sex were not statistically significant between cases and controls (Table 1). There were also no statistically significant differences between cases and controls in the frequency distribution of cigarette smoking, alcohol drinking, *H pylori* infection. However, daily tea drinking appeared to be a protective factor against gastric cancer ($P = 0.035$) and the family history of gastric cancer appeared to be a risk factor ($P = 0.001$) (Table 1). All these differences between cases and controls were further controlled in the multivariate logistic regression analysis.

Table 1 Distribution of selected characteristics of gastric cancer patients and cancer-free controls

Variable	Cases (n = 206)		Controls (n = 261)		P value[1]
	n	%	n	%	
Age (yr)					
≤60	89	43.2	114	43.7	
>60	117	56.8	147	56.3	0.918
Sex					
Male	150	72.8	190	72.8	
Female	56	27.2	71	27.2	0.996
Alcohol status					
Nondrinker	118	57.3	136	52.1	
Drinker	88	42.7	125	47.9	0.265
Smoking status[2]					
Nonsmoker	84	40.8	100	38.6	
Smoker	122	59.2	159	61.4	0.635
H pylori infection[3]					
Negative	91	44.2	93	35.9	
Positive	115	55.8	166	64.1	0.070
Daily drinking of tea					
Yes	77	37.4	123	47.1	
No	129	62.6	138	52.9	0.035
Family history of gastric cancer[4]					
Yes	42	20.5	25	9.6	
No	163	79.5	235	90.4	0.001

[1]Two-side χ^2 test; [2]Two controls missed smoking information; [3]Two controls missed *H pylori* information; [4]One case and one control missed information of family history.

Table 2 CDH1 genotype frequencies of patients and control subjects and their association with gastric cancer

Genotype	Cases (n = 206)		Controls (n = 261)		OR (95%CI)[1]	
	n	%	n	%		
CC	119	57.8	152	58.2[2]	1.00	
CA	75	36.4	91	34.9	1.15	0.78-1.72
AA	12	5.8	18	6.9	0.90	0.40-2.01
	χ^2 = 0.29	P = 0.87				
CC	119	57.8	152	58.2	1.00	
CA+AA	87	42.2	109	41.8	1.05	0.72-1.54
	χ^2 = 0.01	P = 0.92				
C allele	395	75.7	313	76.0	1.00	
A allele	127	24.3	99	24.0	1.02	0.75-1.37
	χ^2 = 0.01	P = 0.92				

[1]ORs were adjusted for age, sex, smoking, alcohol drinking, residence, tea consumption, *H pylori* infection and family history of gastric cancer in a logistic regression model; [2]The observed genotype frequency (CC, CA and AA) in the control subjects was in agreement with Hardy-Weinberg equilibrium (p^2+2pq+q^2 = 1) (χ^2 = 0.738, P = 0.69).

The allele frequency and genotype distribution of *E-cadherin* gene in cases and controls are shown in Table 2. The distribution of genotypes was in agreement with Hardy-Weinberg equilibrium (χ^2 = 0.74, P = 0.69). The genotype frequencies of CC, CA and AA were 57.8%, 36.4% and 5.8%, respectively, in patients, which were very similar to those in controls (58.2%, 34.9% and 6.9%, respectively). Likewise, the A-allele frequencies were 24.0% in cases and 24.3% in controls, respectively. There was no statistically significant difference in CDH1 genotype frequencies and allele frequencies between cases and controls (P = 0.87 for genotype frequency and P = 0.92 for allele frequency). Logistic regression analysis revealed that the variant genotypes CA and AA were not significantly associated with the risk of gastric cancer when compared with the CC wild-type genotype (the adjusted OR [95%CI] was 1.15 [0.78-1.72] for CA heterozygotes and 0.90 [0.40-2.01] for AA homozygotes) (Table 2). There was also no significant association between the combined genotype (CA/AA) and the risk of gastric cancer (CA/AA *vs* CC: adjusted OR = 1.05, 95% CI = 0.72-1.54).

The associations between CDH1 promoter polymorphism and non-cardia gastric cancer stratified on age, sex, smoking and alcohol use, *H pylori* infection and family history of gastric cancer are presented in Table 3. Overall, there was no significant evidence of any associations between the CDH1 genotype and the risk of gastric cancer among these different subgroups in this Chinese population.

DISCUSSION

CDH1 is located on chromosome 16q22.1 and encodes a homophilic transmembrane cellular adhesion protein that is expressed in epithelial tissues. *E-cadherin* acts as a tumor suppressor gene[24] and the dysfunction of CDH1 due to mutations of the gene has been found in diffusive-type gastric cancer[25]. Mutations in CDH1 are the underlying genetic defect in approximately one-third of the hereditary diffuse gastric cancer (HDGC) families. Therefore, CDH1 gene may play an important role in gastric cancer development.

Three lines of evidence have prompted us to further study the association between CDH1 C-160A promoter polymorphism and risk of gastric cancer. First, germ-line mutations in the CDH1 gene predisposes individuals to gastric cancer[26-28]. Second, CDH1 promoter methylation and the associated loss of gene

Table 3 Stratification analyses of CDH1 genotype frequencies, ORs and 95% CIs in gastric cancer

Variables	Cases		Controls		OR (95%CI)[1]
	CC n (%)	CA+AA n (%)	CC n (%)	CA+AA n (%)	
Total	119 (57.7)	87 (42.2)	152 (58.2)	109 (41.8)	
Age (yr)					
≤60	53 (59.6)	36 (40.4)	66 (57.9)	48 (42.1)	0.86 (0.47-1.56)
>60	66 (56.4)	51 (43.6)	86 (58.5)	61 (41.5)	1.20 (0.71-2.00)
Sex					
Male	90 (60.0)	60 (40.0)	107 (56.3)	83 (43.7)	0.92 (0.58-1.44)
Female	29 (51.8)	27 (48.2)	45 (63.4)	26 (36.6)	1.81 (0.85-3.86)
Residence					
Jin-tan	56 (59.0)	39 (41.0)	80 (55.2)	65 (44.8)	0.90 (0.52-1.57)
Huai-an	63 (56.8)	48 (43.2)	72 (62.1)	44 (37.9)	1.31 (0.76-2.27)
Smoking					
No	54 (64.3)	30 (35.7)	58 (58.0)	42 (42.0)	0.79 (0.43-1.48)
Yes	65 (53.3)	57 (46.7)	93 (58.5)	66 (41.5)	1.31 (0.79-2.16)
Alcohol Drinking					
No	71 (60.2)	47 (39.8)	88 (64.7)	48 (35.3)	1.27 (0.75-2.15)
Yes	48 (54.6)	40 (45.5)	64 (51.2)	61 (48.8)	0.92 (0.51-1.63)
H pylori infection					
Negative	54 (59.3)	37 (40.7)	55 (59.1)	38 (40.9)	0.98 (0.53-1.80)
Positive	65 (56.5)	50 (43.5)	95 (57.3)	71 (36.7)	1.03 (0.63-1.71)
Family history of gastric cancer					
No	94 (57.7)	69 (42.3)	138 (58.7)	97 (41.3)	1.07 (0.71-1.62)
Yes	24 (57.1)	18 (42.9)	14 (56.0)	11 (44.0)	1.40 (0.45-4.39)

[1]ORs were adjusted for age, sex, smoking, alcohol drinking, residence, tea consumption, *H pylori* infection and family history of gastric cancer in a logistic regression model.

expression might function as the 'second genetic hit' in the genesis of hereditary diffuse gastric cancer, suggesting that the function of CDH1 gene promoter might play an important role in gastric cancer susceptibility[29]. Third, CDH1 A-160C promoter polymorphism might be a functional polymorphism which could lead to transcriptional downregulation of the gene *in vitro*, and the variant A-allele decreases about 68% transcriptional efficiency compared with C-allele[19]. Therefore this promoter polymorphism might be a potential genetic marker of cancer susceptibility.

To further investigate the association between the functional CDH1 C-160A promoter polymorphism and the risk of non-cardia gastric cancer, we conducted this population-based case-control study in a Chinese population which incorporated information on exposure to smoking, alcohol drinking, *H pylori* infection and other potential confounding factors (age and sex) that were frequency matched between cases and controls and further adjusted in the analysis. However, we did not observe any differences in the distribution of variant genotypes between cases and controls.

Several molecular epidemiological studies have examined the association between the CDH1 promoter polymorphism and the risk of cancers, including prostate and urothelial cancer, as well as breast, colorectal, and gastric cancers, but the results are inconsistent. In a small case-control study of 82 prostate cancer patients and 188 controls, Verhage *et al*[30] reported that CDH1 variant A-allele was associated with a significantly increased risk of prostate cancer in Caucasians (OR = 3.6, 95% CI = 2.0-6.4). Recently, Tsukino *et al*[31] reported that the frequency of CDH1 AA genotype was significantly higher in 314 urothelial cancer patients than in 314 frequency matched healthy controls in Japan (OR = 2.32, 95% CI = 1.03-5.22), the authors' conclusion is that the AA genotype is associated with increased susceptibility to urothelial cancer. However, some other studies failed to find any significant associations between the variant genotype of CDH1 and the risk of breast cancer in Caucasians [32] and colorectal cancer in a British population[33].

Few studies have investigated the association between the CDH1 promoter polymorphism and gastric cancer. Wu *et al*[20] reported that CDH1 A-allele was associated with a significantly reduced risk of gastric cancer (OR = 0.20, 95% CI = 0.06-0.56) compared with CC genotype. However, this study was a hospital-based case-control study and the subjects in Wu's study were from three different ethnic groups. In contrast, in a small case-control study of 53 diffuse gastric cancer patients and 70 cancer-free controls in New Zealand, Humar *et al*[21] reported the OR of 2.27 for CA heterozygotes (95% CI = 1.16-4.44) and 7.84 for AA homozygotes (95% CI = 2.89-21.24) associated with an increased risk of gastric cancer. However, a study combining three small case-control studies in the United Kingdom reported that the genotype frequencies of CDH1 did not differ between 433 gastric cancer patients and 466 cancer-free controls, and this polymorphism was not associated with the risk of gastric cancer[34]. The results from our present population-based case-control study of gastric non-cardia cancer are consistent with those of Pharoah *et al*[34].

One reason for these discrepancies in the above studies may be the difference in genetic polymorphisms between different ethnic groups. Another reason might be due to different sites and different pathological types of gastric cancer. In addition, there might be a difference in the etiology of the cardia and non-cardia gastric cancers and between intestinal- and diffuse-type gastric cancers. In this study, we did not find any association between this CDH1 genotype and the risk of non-cardia gastric cancer. Moreover, because we lacked histologic information for all gastric cancer cases, we did not perform the analysis on the association between CDH1 polymorphism and risk of gastric cancer in different histopathologic subgroups. Therefore, further studies are needed to confirm our results.

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