RESEARCH ARTICLE

The *COX-2* -765 G>C Polymorphism is Associated with Increased Risk of Gastric Carcinogenesis in the Chinese Hui Ethnic Population

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Abstract

Background: The Chinese Hui ethnic group has diverse origins, including Arab, Persian, Central Asian, and Mongol. The standardized mortality rate of gastric cancer in the Hui population is higher than the overall Chinese population. In this study, we investigated whether *COX-2*-765G>C polymorphism, an extensively studied polymorphism, contributes to gastric cancer and its precursor lesions (GPL) in the Chinese Hui ethnic group. Materials and Methods: *COX-2*-765G>C polymorphism was determined by pyrosequencing in 100 gastric cancer cases, 102 gastric cancerand its precursor lesions cases and 105 controls. Data were statistically analyzed using Chi-square tests and logistic regression models. Results: Among the Chinese Hui ethnic group *COX-2* -765 C allele carriers were at increased risk for gastric cancer (OR=1.977, 95%CI=1.104-3.541). We also found an interaction between *COX-2* -765 C carriers and *Helicobacter pylori* infection and eating pickled vegetables. Conclusions: Our findings suggest a multi-step process of gene-environment interaction contributes to gastric carcinogenesis.

Keywords: Cyclooxygenase-2 - gastric cancer - gastric precursors lesions - polymorphisms - *Helicobacter pylori*

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Introduction

Gastric cancer is a major health problem, being the most common malignancy in the world with an estimated one million new cases every year (Hartgrink et al., 2009). The high morbidity areas are found in Asia, especially China. It is reported that the standardized mortality rate of gastric cancer in Hui population is higher than the averag elever of China (The Data-Center of China Public Health Science). In China, Hui minority ethnic populations are descendant of Mongols and Muslims immigrants. Through interracial breeding between the Han, the Mongols, and the Uygur, they developed the Hui nationality. There are many concentrated Hui communities in Gansu province where there are high incidence areas of gastric cancer. Our previous study suggests *COX-2* plays an important role in carcinogenesis and its polymorphisms, including a new single nucleotide polymorphism (SNP) are associated with susceptibility to gastric cancerin the Han population in Gansu Province (Li et al., 2006; Li et al., 2010; Zhu et al., 2010). To the best of our knowledge, there are no other reports on the SNPs of *COX-2* and its association with increased gastric cancer risk in the Chinese Hui population. In this study, we investigated whether the *COX-2*-765G>C polymorphism contributes to gastric cancer in the Chinese Hui ethnic population.

Materials and Methods

Study population

Subjects investigated were Muslim inhabitants, who lived in Linxia Hui Autonomous Prefecture for more than 20 years with three generations of non-ethnic marriage. The case control study was undertaken between October 2010 to November 2012, and consisted of 100 gastric cancer patients, 102 precancerous lesion patients and 105 control subjects. Participants were recruited from2A grade hospitals in Gansu Province. The diagnosis of gastric cancer and precancerous lesion were on the basis of clinical, endoscopical and histopathological examinations. The 105 control subjects were confirmed to be free of the disease by a health examination, involving endoscopy or upper gastrointestinal investigation.Subjects were informed of the detailed study protocol,and signed consent forms, and the study was approvedby local ethics committees.

A questionnaire collected information on (a) demographic factors, such as age, sex, ethnic, native place; (b) medical history of digestive disease and family history of gastric cancer. Subject with at least one first-degree relative (a parent or sibling) or two second-degree relatives (grandparents, aunts, or uncles) with gastric cancer (GC) were considered to have a positive family

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history. (c)frequency of eating pickles (at least 5 times a week or more).

Each subject donated 3 mL peripheralvein blood in EDTA-K2 anticoagulative tube for DNA extraction and 2 mL serum for *H. pylori* infection test, and all specimens were kept frozen at -80°C.

Sample DNA extraction

Blood samples were collected with a standard venipuncture technique using EDTA containing tubes. Genomic DNA was extracted using EZ Spin Column Genetic DNA Isolation Kit (BBI, Canada) following the manufacturer's instruction. The integrity and purity were determined by using agarose gel electrophoresis and a spectrophotometer respectively. The final concentration of each DNA sample was adjusted to approximately 20 ng/mL and stored at -80°C until use.

*Detection of **H. pylori** infection*

Presence and type of *H. pylori* infection were tested using a *H. pylori* antibody Immunoblotting Kit (Blot Biotech,Shenzhen, China) following the manufacturer's instructions. Type I *H. pylori* infection was defined when either CagA or VacA was positive, or both were positive. TypeII *H. pylori* infection was defined if ureases (UreA/UreB)were positive. Patients were defined as *H. pylori* negative if CagA, VacA and ureases were negative.

*Genotyping of **COX-2**-765G>C polymorphism*

COX-2-765G>C polymorphismwas determined by pyrosequencing technology. PCR primers and sequencing primer were designed by TakaraBioTechnology (Dalian) Corporation Limited. The sequences of PCR primers were as follows: forward primer was TTTATATTGGTGACCCGTGGAGC, and reverse primer was biotinylated-CCTTCACCCCCTCCTTGTTT. The sequencing primer was GGAGAATTTACCTTTCCC. All primers were validatedby basic local alignment search tool (BLAST) homology searches (http://www.ncbi.nlm.nih.gov).

Quality control

Twenty percent of samples from patients and controlswere regenotyped by other laboratory personnel, and results showed 100% similarity in both of the conditions. No discrepancy was found after sequencing randomly selecting 10% of the samples.

Statistical analysis

The Hardy-Weinberg equilibrium equation was used to determine whether the proportion of each genotype obtained was in agreement with expected values as calculated from allele frequencies. Student and Chi-square test analyses was used to compare categorical variables and genotype frequencies between GC, GPL and controls, using a 5% level of significance. Odds ratios (ORs) and 95% confidence intervals (CIs) calculated bynon-conditional logistic regression analysiswas performed to analyze the association betweenthe genotypes of *COX-2* and the risk factors ofGC. Multivariate logistic regression was used to analyze all risk factors with GC and GPL.

Table 1. Demographic Characteristics and Risk Factors in Subjects with Gastric Cancer or GPL, and Control Subjects

Variable	Control (n=105)	GPL (n=102)	Gastric cancer (n=100)	*p*-Valuea
Age	58.3±11.4[b]	58.1±11.3	58.5±11.2	0.922
<60[n (%)]	45(42.9)	44(43.1)	42(42.0)	
≥60[n (%)]	60(57.1)	58(56.9)	58(58.0)	
Sex				0.953
Female	34(32.4)	33(32.4)	32(32.0)	
Male	71(67.6)	69(67.6)	68(68.0)	
H. pylori infection [n (%)]				0.039
Yes	52(49.5)	65(63.7)	67(67.0)	
No	53(50.5)	37(36.3)	33(33.0)	
Family history [n (%)][c]				0.028
Yes	12(11.4)	15(14.7)	23(23.0)	
No	93(88.6)	87(85.3)	77(77.0)	
Eating pickle vegetables [n (%)][d]				0.023
Yes	23(21.9)	37(36.3)	42(42.0)	
No	82(78.1)	65(63.7)	58(58.0)	

*a: *p*-Values from Student's t-test and chi-squared test, b: Mean ±SD,c: At least one first-degree relative (a parent or sibling) or two second-degree relatives (grandparents, aunts, or uncles) with gastric cancer, d: Continuous history of 10 years or more, no less than 3 months per year

Allele frequency distribution was stratified by age, sex, GC family history, eating pickles history and *H. pylori* infection, and interactions were considered in the model. All data analysis was performed using the computer software SPSS 13.0 (SPSSInc., Chicago, IL, USA).

Results

Demographic and clinical characteristics

The frequency distribution of sex, age, *H. pylori* infection, family history and eating pickle vegetables in subjects with gastric cancer, GPL and controls is presented in Table 1. Rates of *H. pylori* infection and eating pickled vegetables were significantly higher in gastric cancer and GPL participants compared to controls whereas no significant differences were seen between gastric cancer and GPL. The rates of gastric cancer family history was significantly higher in gastric cancer and GPL participants compared to controls. The was nosignificant differences in age and sex between gastric cancer and GPL participantscompared to controls.

*Genotype frequencies of **COX-2** -765G>C polymorphism*

The distribution of *COX-2* -765G>C polymorphism was consistent with Hardy-Weinberg's equilibrium in healthy controls. However, the *COX-2*-765 CC genotype significanlty increased the risk for gastric cancer (OR=2.400) compared to the GG genotype (p=0.029) (Table 2).

*Stratification analysis of **COX-2** -765 G>C polymorphism*

The risk of GPL or gastric cancer related to *COX-2* -765G>C genotypes were further examined with stratificationby *H. pylori* infection, family history and eating pickled vegetables (Table 3). The intake of pickled vegetables was the most importantrisk factor. The presence of the -765 C allele in subjects with eating pickle vegetables conferred a 3-fold risk of gastric cancer compared with GG genotypes. However, individuals carried C allele appeared to have no association between

Table 2. Genotype Frequencies of *COX-2*-765 G > C Polymorphisms in Gastric Cancer, GPL and Control Subjects

Genotype	Control(n=105) (%)	GPL (n=102) (%)		Gastric cancer (n=100) (%)	
		n (%)	OR (95% CI)	n (%)	OR (95% CI)
GG	76 (72.4)	64 (62.7)	1	55 (55.0)	1
GC	24 (22.9)	32 (31.4)	1.583 (0.847, 2.958)	11 (11.0)	1.889 (1.011, 3.530)
CC	5 (4.7)	6 (5.9)	1.425 (0.415, 4.888)	34 (34.0)	2.400 (1.763, 7.549)*
GC+CC	29 (27.6)	38 (37.3)	1.556 (0.865, 2.798)	45 (45.0)	1.977 (1.104, 3.541)*

*CI, confidence intervals; GPL, gastric precancerous lesion; OR, Odds ratio; *p<0.05

Table 3. Correlation of *COX-2*-765 G > C Genotypes with Susceptibility to GPL, Gastric Cancer, Stratified by Risk Factors

Risk factors	Control (n=105)		GPL (n=102) (%)			Gastric cancer (n=100) (%)		
	GG (%)	GC+CC (%)	GG (%)	GC+CC (%)	OR1 (95%CI)[a,b]	GG (%)	GC+CC (%)	OR2 (95%CI)[a,c]
H.pylori infection								
Yes	35 (33.3)	17 (16.2)	36 (35.3)	29 (28.4)	1.658 (0.777, 3.540)	33 (33.0)	34 (34.0)	2.121 (1.020, 4.498)*
No	41 (39.1)	12 (11.4)	28 (27.5)	9 (8.8)	1.098 (0.409, 2.952)	24 (24.0)	9 (9.0)	1.281 (0.471, 3.484)
Family history								
Yes	7 (6.7)	5 (4.8)	8 (7.8)	7 (6.9)	1.225 (0.265, 5.667)	10 (10.0)	13 (13.0)	1.486 (0.402, 5.491)
No	69 (65.7)	24 (22.8)	56 (54.9)	31 (30.4)	1.592 (0.840, 3.015)	47 (47.0)	30 (30.0)	1.153 (0.611, 2.174)
Eating pickle vegetables								
Yes	14 (13.3)	9 (8.6)	17 (16.7)	20 (19.6)	1.830 (0.635, 5.271)	16 (16.0)	26 (26.0)	3.381 (1.563, 3.390)*
No	62 (59.0)	20 (19.1)	47 (46.1)	18 (17.6)	1.187 (0.566, 2.491)	41 (41.0)	17 (17.0)	1.083 (0.494, 2.371)

*[a]ORs and 95% CIs were calculated by logistic regression, with the CC genotype as the reference group, and adjusted for age and sex, [b]OR1 is for the comparison CG+GG *vs* CC genotype for GPL, [c]OR2 is for the comparison CG+GG *vs* CC genotype for gastric cancer, CI, confidence interval; GPL, gastric precancerous lesion; OR, odds ratio; *p<0.05

GPL and controls (OR=1.830, 95%CI=0.635-5.271, p=0.261). In *H. pylori* positive groups, the risk of gastric cancer was significantly higher in subjects who carried C allele compared with controls (OR=2.121, 95%CI=1.020-4.498, p=0.048). While there was no association between GPL and controls among *H. pylori* positive participants (OR=1.658, 95%CI=0.777-3.540, p=0.190). There was no increased risk amongst participants with a family history of gastric cancer in all three groups.

Discussion

Living habits of the Hui minority have a deep Islamic influence. As a result smoking and drinking, the two major risk factors for gastric cancer (Yaegashi et al., 2014), are forbidden in their daily lives. However, the Hui minority in Northwest China like to eating pickled vegetables. Our study demonstrated the intake of pickle vegetables was the highest risk factor for gastric cancer in the Hui population which has been shown in other studies (Ren et al., 2012). *H. pylori* are also a proven risk factor for development of gastric cancer (Uemura et al., 2001).

In this study, we found *H. pylori* infection increased the risk of gastric diseases, but there was no different between gastric cancer and GPL groups. This finding suggests that *H. pylori* infection didn't aggravate inflammation reactions, and was only an early event during of development of gastric cancer. Some studies investigating the effect of *H. pylori* eradication have shown contradictory results for reversibility of precursor lesions and reduction of gastric cancer rate (Kuipers and Sipponen., 2006). Therefore, *H. pylori* eradication was a good prophylactic method but clinical way.

Gene polymorphisms vary substantially between ethnic groups. As a results it is important to identify the susceptible genes in different ethnicity. *COX-2* is a rate-limiting enzyme that catalyzes the formation of prostaglandins (PG) from arachidonic acid (AA). *COX-2* -765 G>C is one of the most extensively studied polymorphisms. It has been reported that the -765 CC genotype does not exist in Han population (Liu et al., 2006; Tan et al., 2007). However, we found the frequency of genotype was about 5% -765 G>C increased the risk of gastric cancer in the Han population. Pereira et al. found similar results amongst Portuguese populations (Pereira et al., 2006). However, Sitarz et al. findings suggest the polymorphism plays a protective role in the development of gastric cancer (Sitarz et al., 2008). Interestingly, they founded there was no correlation between the presence of the C allele and a difference in *COX-2* expression. In addition, this polymorphism showed no associations with gastric cancer and precancerous lesions risk in Chinese Han people.[9] Therefore, it might indicate that the -765 G>C polymorphism plays a role in carcinogenic processes in specific populations.

The present study has some limitations. The sample size was relatively small, although it was sufficient to detect moderate associations. In addition, the risk of GC related to the *COX-2* SNP might be influenced by use of non-steroidal anti-inflammatory drugs. Those factors might interact with *COX-2* genotype or act as potential confounder in the analysis. Unfortunately, information on this factor in the present study was unavailable. It would be important in the future to investigate the interaction between the genotype and the expression *in vitro*.

In summary, this study provides evidence that the *COX-2*-765 G>C polymorphism increased the genetic susceptibility of gastric cancer in Hui population. We also found an interaction among *H. pylori* infection, eating pickle vegetablesand the *COX-2* -765 C carriers.

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