RESEARCH ARTICLE

SNPs of Excision Repair Cross Complementing Group 5 and Gastric Cancer Risk in Chinese Populations

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Abstract

We conducted a case-control study to determine the association between several potential SNPs of excision repair cross complementing group 5 (XPG) and gastric cancer susceptibility, and roles of XPG polymorphisms in combination with *H.pylori* infection in determining risk of gastric cancer. In our study, we collected 337 newly diagnosed gastric cancer cases and 347 health controls. Three SNPs of XPG, rs2296147T>C, rs2094258C>T and rs873601G>A, were genotyped using the Taqman real-time PCR method with a 7900 HT sequence detector system. *H. pylori* infection was diagnosed by ELISA. By multivariate logistic regression analysis, the rs2296147 CC genotype was associated with a decreased risk of gastric cancer (OR=0.52, 95% CI=0.27-0.97), and rs2094258 TT was associated with elevated risk (OR=2.13, 95% CI=1.22-3.35). Positive *H.pylori* individuals with rs2094258 TT genotypes demonstrated increased risk of gastric cancer (OR=2.13, 95% CI=1.22-3.35), while rs2296147 CC was associated with lower risk among patients with negative *H.pylori* (OR=0.45, 95%CI=0.22-0.89). Our findings suggested that XPG polymorphisms might contribute to risk of gastric cancer among Chinese populations, but the effect needs to be further validated by larger sample size studies.

Keywords: XPG - SNP - gastric cancer - *H.pylor*i - Chinese populations

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Introduction

Worldwide, about one million new cases of stomach cancer were estimated to have occurred in 2008 (988 000 cases, 7.8% of the total), making it currently the fourth most common malignancy in the world, behind cancers of the lung, breast and colo-rectum. More than 70% of cases occur in developing countries, and half the world total occurs in China (IARC, 2008). The wide geographic variation at international levels of gastric cancer in terms of incidence indicated that the genetic and environmental factors may play a role in pathogenesis of this cancer.

Helicobacter pylori, which has been classified as group I carcinogen by World Health Organization, is recognized as one of the most important risk factors for gastric carcinogenesis (Correa, 1992; IARC, 1994). Almost 50% to 80% of the world's population infected with H.pylori, but only 1% of them developed gastric cancer (Graham et al., 1991; Parsonnet et al., 1997), which suggested the host genetic and environmental factors may modulate the risk of gastric cancer in association with H.pylori infection (Singh et al., 2006; Ghoshal et al., 2008; Ghoshal et al., 2007).

DNA repaired genes play a crucial role in maintaining the stability and integrity of genomic DNA. The Excision repair cross complementing group 5 (XPG or ERCC5) gene encodes an 1186-amino acid structure-specific endonuclease that is essential for the two incision steps in NER (Mueser et al., 1996; Friedberg et al., 2000). The additional functions of XPG are evident by the presence of structural motifis that are common with proteins involved in repair-recombination and cell cycle regulation (Klungland et al., 1999). It is reported that polymorphisms in XPG are associated with various cancers, such as colorectal cancer, esophageal cancer and lung cancer as well as bladder cancer (Chang et al., 2008; Pan et al., 2009; Rouissi et al., 2011; Liu et al., 2012). However, there was only one study conducted in Chinese population about the association between XPG and gastric cancer risk (He et al., 2012). This study suggested XPG was associated with risk of gastric cancer

In our study, we conducted a case-control study to determine the association between several potential SNPs of XPG and gastric cancer susceptibility, and role of XPG polymorphisms in combination with H.pylori infection in the risk of gastric cancer.

Materials and Methods

This case-control study included 343 patients with newly diagnosed and histopathologically confirmed primary gastric cancer, including gastric cardia adenocarcinoma

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and non-gastric cardia adenocarcinoma. All the cases were selected from the First Affiliated Hospital of Zhengzhou University between January 2009 and December 2010. Case with secondary or recurrent tumors was excluded, and finally 337 patients were included. Hospital-based controls were individually matched to case-patients by gender and age (±5 years). Controls were patients selected from Surgical Department, Plastic Surgery Department, ENT Department and Department of Gynecology. Totally, we had 352 controls that were malignant tumors or digestive tract disorders free, and finally 347 health controls would like to participate into our study.

All the cases and controls signed the formed consent and provided 3 ml blood for genomic DNA extraction. Demographic data and environmental exposure history were collected, including age, sex, smoking status and alcohol status.

H.pylori infection Diagnosis

The H. pylori infection was diagnosed by enzyme linked immunoabsorbent assay (ELISA) for IgG antibodies (HpIgG ELISA) using commercially available kit (Genesis Diagnostics, Cambridgeshire, UK) as per manufacturer's instructions on sera obtained from 5 mL blood. The sensitivity and specificity of the kit was 91% and 100%, respectively.

SNP selection and genotyping

Genomic DNA was extracted from the buffy coat fraction of each blood sample by using a Qiagen Blood Kit (Qiagen, Chastworth, CA) according to the manufacturer's protocolinstructions. DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 UV spectrophotometer. The potentially SNPs of XPG of interest were selected from NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/) and SNPinfo (http://snpinfo.niehs.nih.gov/). SNPs located at two ends of XPG gene, and with the minor allele frequency (MAF) less than 5% of Chinese people, and influencing the microRNA binding sites activity. Finally, three SNPs were included in our study, including rs2296147T>C, rs2094258C>T and rs873601G>A. All the three SNPs were genotyped using the Taqman real-time PCR method with a 7900 HT sequence detector system (Applied Biosystems, Foster City, CA). To ensure high genotyping accuracy, strict quality control procedures were implemented, and four duplicated positive controls and four negative controls (no DNA) were used in each of 384-well plates. Approximately 5% of the samples were repeatedly genotyped, and the results were 100% concordant.

Statistical analysis

The descriptive data for the major characteristics of study groups are expressed as mean and percent. Differences in frequency distributions of the selected demographic variables, risk factors and each of alleles and genotypes of the selected SNPs between patients with gastric cancer and control subjects were evaluated by using the χ^2 test. We compared differences in genotype distribution of rs2296147T>C, rs2094258C>T and rs873601G>A among cases and controls, as well as tests for Hardy-Weinberg equilibrium in controls. Adjusted odds ratios (OR) and their 95% confidence intervals (95% CI) of the association between genotype and gastric cancer were calculated by multivariate logistic regression models with adjustments for potential confounding factors, including sex, age, smoking, drinking and H.pylori. The interaction of genotype with smoking and H.pylori and smoking was estimated by using the logistic regression model. Statistical analysis was performed by using SPSS version 16.0 statistical software (SPSS, Chicago, IL, USA). We used t-tests to determine statistical differences in the continuous variables and chi-square test for the categorical variables. Statistical significance was set at $P<0.05$ and all tests were two-sides.

Results

Population characteristics

The final analysis included 337 cases of gastric cancer and 347 controls, whose characteristics are summarized in Table 1. There was no significant difference in the distributions of age and sex between the cases (mean age of 58.6±7.5 years and 59.1±7.8 years) and controls. However, cases were more likely to be smokers and drinkers, and cases were more likely to be infected with H.pylori.

Distribution of XPG SNPs and association with gastric cancer risk

The allele and genotype frequencies of the three candidate SNPs in cases and controls were summarized in Table 2. All the observed genotype frequencies agreed with the Hardy-Weinberg equilibrium in the control (P=0.15 for rs2296147, P=0.35 for rs2094258 and P=0.24 for rs873601). The rs2296147 CC genotype was more frequent among cases than controls, whereas the rs2094258 TT genotype was less frequent among cases than controls, and significant difference was found (P=0.03 for rs2296147 and P=0.02 for rs2094258). In the single factor analysis, rs2296147 CC and rs2094258 TT found significant association with gastric cancer risk, with OR and 95% CI of 0.55(0.31-0.98) and 1.75(1.06-2.92),

Table 1. Distributions of Demographic Characteristics

Characteristics	Cases N=337(%)	%	Controls N=347(%)	%	χ^2	P
Age, yr (Mean±SD)	58.6±7.5		59.1±7.8			
<50	60	17.8	57	16.43		
50~65	207	61.42	216	62.25	0.23	0.89
>65	70	20.77	74	21.32		
Sex						
Males	194	57.57	196	56.48		
Females	143	42.43	151	43.52	0.82	0.78
Smoking status						
Ever	143	42.43	120	34.58	4.45	0.03
No	194	57.57	227	65.42		
Drinking status						
Ever	134	39.76	110	31.7		
No	203	60.24	237	68.3	4.84	0.03
H.pylori infection						
Positive	211	62.61	189	54.47		
Negative	126	37.39	158	45.53	4.67	0.03

Table 2. Association Between XPG SNPs and Gastric Cancer Risk

Genetic polymorphisms	Cases N=337	%	Controls N=347	%	OR (95% CI)	P	Adjusted OR (95% CI)[1]	P
rs2296147								
TT	208	61.72	196	56.48	1.0(Ref.)	-	1.0(Ref.)	
CT	105	31.16	110	31.7	0.90(0.64-1.27)	0.53	0.93(0.66-1.36)	0.49
CC	24	7.12	41	11.82	0.55(0.31-0.98)	0.03	0.52(0.27-0.97)	0.02
CT+CC	129	38.28	151	43.52	0.81(0.59-1.11)	0.16	0.76(0.53-1.03)	0.14
rs2094258								
CC	131	38.87	145	41.79	1.0(Ref.)	-	1.0(Ref.)	
CT	149	44.21	166	47.84	0.99(0.71-1.39)	0.96	0.98(0.69-1.41)	0.84
TT	57	16.91	36	10.37	1.75(1.06-2.92)	0.02	1.81(1.14-3.10)	0.02
CT+TT	206	61.13	202	58.21	1.13(0.82-1.55)	0.44	1.21(0.80-1.64)	0.37
rs873601								
GG	96	28.49	91	26.22	1.0(Ref.)	-	1.0(Ref.)	
AG	163	48.37	164	47.26	0.94(0.65-1.37)	0.75	0.96(0.68-1.41)	0.8
AA	78	23.15	91	26.22	0.81(0.52-1.26)	0.33	0.83(0.57-1.35)	0.37
AG+AA	241	71.51	256	73.78	0.89(0.63-1.27)	0.51	0.85(0.67-1.33)	0.46

[1]Adjusted for sex, age, drinking, smoking and H.pylori infection

Table 3. Modification Effect of H.pylori infection on the Association Between XPG SNPs and the Risk of Gastric Cancer

Genetic	H.pylori infection			
	Positive		Negative	
polymorphisms	OR (95% CI)[1]	P	OR (95% CI)[1]	P
rs2296147				
TT	1.0(Ref.)	-	1.0(Ref.)	-
CT	1.03(0.66-1.56)	0.64	0.85(0.60-1.24)	0.21
CC	0.74(043-1.21)	0.01	0.45(0.22-0.89)	0.01
rs2094258				
CC	1.0(Ref.)	-	1.0(Ref.)	-
CT	1.02(0.83-1.55)	0.85	0.92(0.56-1.53)	0.67
TT	2.13(1.22-3.35)	0.01	1.68(0.98-2.94)	0.08
rs873601				
GG	1.0(Ref.)	-	1.0(Ref.)	-
AG	1.07(0.77-1.48)	0.66	0.87(0.63-1.38)	0.65
AA	0.95(0.59-1.34)	0.27	0.77(0.53-1.40)	0.25

[1]Adjusted for sex, age drinking and smoking

respectively. After adjustment for age, sex, drinking, smoking and H.pylori infection, rs2296147 CC genotype was associated with a decreased risk of gastric cancer (OR=0.52, 95% CI=0.27-0.97), and rs2094258 TT was associated with higher risk of gastric cancer (OR=1.81, 95% CI=1.14-3.10).

Interaction association between SNPs of XPG and H.pylori

Stratification was conducted regarding SNPs of XPG and H.pylori in Table 3. We found H.pylori modified the association between rs2296147 and rs2094258 genotypes and the risk of gastric cancer. That is, positive H.pylori individuals with rs2094258 TT genotypes have higher increased risk of gastric cancer (OR=2.13, 95% CI=1.22-3.35, P for interaction=0.03), while rs2296147 CC was associated with lower risk of gastric cancer among patients with negative H.pylori (OR=0.45, 95%CI=0.22-0.89, P for interaction=0.01). However, we did not find a significant modification of H.pylori in the association between rs873601 and cancer risk.

Discussion

In the present case-control study, we investigated the association between the three common, putatively functional SNPs of the XPG gene and the risk of gastric cancer in center Chinese population. We found the rs2296147 CC was associated with decreased risk of gastric cancer, and rs2094258 TT significantly increased gastric cancer risk, but this risk was not observed in rs873601G>A SNP. Moreover, we found the H.pylori modified the association between SNPs and gastric cancer risk. To our best knowledge, it is the first study to investigate the association between SNPs and gastric cancer risk in center Chinese population, and the modification role of H.pylori on their association.

XPG is responsible for a 1186 amino acid structure-specific endonuclease, the activity of which is essential for the 2 incision steps in NER. In human cells, XPG catalyzes an incision approximately 5 nucleotides 3' to the site of damage and is also involved non-enzymatically in the subsequent 5' incision (Kiyohara et al., 2007). It is further involved in the stabilization of a pre-incision complex on the damaged DNA. It has only several studies reported the association of the SNPs of XPG and risk of cancers in Chinese population (Duan et al., 2012; Ma et al., 2012; Zhu and Shi et al., 2012). A study conducted in east China indicated rs2296147 CC was associated with decreased risk of esophageal cancer, of which was conducted among 1,115 squamous cell carcinoma cases and 1,117 cancer-free controls (Zhu and Shi, 2012). Another study included 403 cases and 403 health controls indicated rs751402 and rs2296147 polymorphisms might alter the risk of developing gastric cancer, and especially the diffuse subtype (Duan et al., 2012). A case-control study with 1059 cases and 1066 cancer free controls did not find a significant association with head and neck cancer risk (Ma et al., 2012). A meta-analysis study with 23,490 cases and 27,168 controls indicated polymorphisms in XPG have role in various cancer risk, including skin cancer, breast cancer and smoking-related cancers (Zhu and Wang et al., 2012). In our study, we found rs2296147T>C and rs2094258C>T were associated with gastric cancer risk, and only one case-control study showed rs2296147C>T was associated with this cancer risk, and did not find association of other genotypes (Duan et al., 2012). The possible discrepancy of the results of XPG polymorphisms in gastric cancer may be due to different backgrounds of cases, sample size, sample size and etc. Therefore,

the association between XPG SNPs with gastric cancer risk needs to be validated in additional large sample size studies of different homogenous ethnicity or more ethnically diverse groups.

H.pylori has been implicated in gastric carcinogenesis (Sipponen and Marshall, 2000). It may be a good model of inflammation-associated carcinogenesis, because H. pylori infection induces intense acute and chronic inflammatory reaction that may last for decades (Price, 1991; Dixon et al., 1996). Moreover, H.pylori infection, environmental exposures, host genetic susceptibility, or some combination of these factors can lead to increased expression of cytokines, growth factors and their receptors, which in turn promotes cellular proliferation (Coussens and Werb, 2002; Lu et al., 2006). In addition, free radicals are generated as by-products of the inflammatory response. An important consequence of increased cellular proliferation and presence of free radicals is DNA damage, which can predispose an individual to cancer pathogenesis. Previous study indicated H.pylori infection may directly mutate cellular DNA in the gastric epithelium, and thus modify the association between DNA repaired gene and gastric cancer risk (Farinati et al., 2008; Hussain et al., 2009). Our study indicated H.pylori could modify the association between polymorphisms in XPG and gastric cancer risk, which was in line with previous studies.

Some limitations should be considered in our study. Firstly, although our study was relatively large, the small sample size in subgroup analysis may have limited statistical power to find the difference between gene-gene and gene-environment interactions adequately. Second, we only analyzed the association between some potential SNPs and gastric cancer risk, which might miss some important genetic variations within the gene. Finally, our study is a hospital-based case-control study, and this population may not represent other Chinese population. Selection bias may also exist.

In summary, our findings suggested that XPG polymorphisms might contribute to risk of gastric cancer among center Chinese populations, but the effect needs to be further validated by larger sample size studies.

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