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Association between *ERCC5* gene polymorphisms and gastric cancer risk

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ABSTRACT. We investigate the role of *ERCC5* gene polymorphisms (rs17655 and rs751402) in the development of gastric cancer in a Chinese population. A total of 142 gastric cancer patients whose diagnoses were confirmed by pathology, and 274 control subjects were recruited from Tangshan Gongren Hospital between March 2013 and March 2015. Genotyping of *ERCC5* rs17655 and rs751402 polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism. Compared with the control subjects, we found that gastric cancer patients were more likely to be older, smoke tobacco, drink alcohol, and suffer from *Helicobacter pylori* infection. Using a chi-square test, a significant difference was observed in the distribution of*ERCC5* rs751402 genotypes between patient and control groups (chi-square = 7.79, P = 0.02). In addition, unconditional multiple logistic regression analysis revealed that the AA genotype of rs751402 significantly increased gastric cancer risk compared to the GG genotype [odds ratio (OR) = 2.61, 95%CI = 1.23-5.49; P = 0.005]. Moreover, we found that the AA genotype correlated with elevated risk of gastric cancer when compared to the GG+AG genotype under a recessive

model (OR = 2.21, 95%CI = 1.11-4.39; P = 0.01). In conclusion, we suggest that the *ERCC5* rs751402 polymorphism is associated with development of gastric cancer.

Key words: *ERCC5*; Polymorphism; Gastric cancer

INTRODUCTION

Gastric cancer is one of the most frequently observed malignancies globally. In 2012, an estimated 952,000 cases and 723,000 gastric cancer-related deaths were reported, accounting for 6.76% of the total number of cancer cases and 8.81% of all cancer mortalities (Jemal et al., 2011; Ferlay et al., 2013). Gastric cancer incidence rates are highest in developing countries, with half of all cases occurring in Eastern Asia (principally China). *Helicobacter pylori* infection is reported to be a risk factor in the development of gastric ulcers and cancer. Previous studies have indicated that certain lifestyle characteristics influence gastric oncogenesis, such as alcohol consumption, obesity, and high sodium intake (van den Brandt and Goldbohm, 2006). However, not all individuals subjected to risk factors such as these go on to develop gastric cancer, which implies that genetic components also influence susceptibility to this disease.

It is well known that excision repair cross-complementing rodent repair deficiency, complementation group 5 (ERCC5) is a key member of an enzyme family incorporating the DNaseIV/flap structure-specific endonuclease 1 group of structure-specific nucleases, and plays a role in nucleotide excision repair (NER; Hohl et al., 2007). Previous studies have reported that *ERCC5* gene polymorphisms are associated with the development of certain malignancies, including colorectal, breast, lung, laryngeal, and prostate cancers, and glioma (Luo et al., 2013; Liang et al., 2014; Lu et al., 2014; Zhang et al., 2014; Na et al., 2015; Zeng et al., 2015). Few studies have reported the association between *ERCC5* gene polymorphisms and gastric cancer risk (Hussain et al., 2009; Duan et al., 2012). In this study, we performed a case-control study to investigate the role of *ERCC5* genetic variations (rs17655 and rs751402) in development of gastric cancer in a Chinese population.

MATERIAL AND METHODS

Subjects

A hospital-based case-control design was used for this study. A total of 142 gastric cancer patients with pathologically confirmed diagnoses were recruited from Tangshan Gongren Hospital between March 2013 and March 2015. All diagnoses were confirmed for the purposes of this study. Individuals with secondary or recurrent tumors, or a history of other tumors were excluded from this investigation.

Control participants were randomly selected from Tangshan Gongren Hospital between March 2013 and March 2015. A total of 274 gastric cancer-free individuals were recruited during regular health examinations in our hospital. Written informed consent was obtained from all participants, and our study conformed to the standards of the Declaration of Helsinki.

The lifestyle and clinical characteristics of gastric cancer patients and control subjects were ascertained from medical records, and included sex, age, tobacco and alcohol consumption, and tumor stage. *Helicobacter pylori* infection was diagnosed using serology tests.

DNA extraction and genotyping

The DNA was extracted from the peripheral blood using aTIANamp Blood DNA Kit (Tiangen, Beijing, China) following the manufacturer protocol. The extracted DNA was kept at -20°C until needed. Genotyping of *ERCC5* rs17655 and rs751402 was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism. Details of primers, restriction digest products, and restriction enzymes are given in Table 1. Cycling conditions were 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 45s, and a final extension at 72°C for 7 min.

Table 1. Primers, restriction enzymes, and restriction digest products used to genotype *ERCC5*rs17655 and rs751402.

Polymorphism	Primers	PCR product length	Restriction enzyme
rs17655	5'-CATCTGATGGATCTTCAAGTCTA-3' 5'-TCACGAGGACCATCTTCT-3'	314 bp	*Xba*I
rs751402	5'-GAGCGGGCCCATTTTCC-3' 5'-TCACCGCCTCCCGGAAGAAAGA-3'	218 bp	*Nco*I

PCR = polymerase chain reaction.

Statistical analysis

Differences in lifestyle characteristics and genotype distributions between patient and control groups were analyzed by chi-square test. Univariate logistic regression analysis was used to estimate the relative risk conferred by lifestyle factors in both groups. Hardy-Weinberg equilibrium (HWE) was examined using a chi-square test with one degree of freedom. Multiple logistic regression analysis was employed to assess the association between *ERCC5* rs17655 and rs751402 gene polymorphisms and gastric cancer risk. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated to assess the results. All analyses were conducted with SPSS statistical software, version 17.0 (SPSS Inc., Chicago, IL, USA). All tests were two-sided with a significance level of $P < 0.05$.

RESULTS

This study included 54 and 128 women and 88 and 146 men in the patient and control groups, respectively (Table 2). The mean ages of patients and controls were 64.75 ± 12.50 and 61.38 ± 11.83 years, respectively. Compared to control subjects, we found that gastric cancer patients were more likely to be older, smoke tobacco, drink alcohol, and suffer from *Helicobacter pylori* infection. Regarding clinical categories, 73 (51.41%) patients were at stages I-II, while 69 (48.59%) suffered cancers of stages III-IV.

ERCC5 rs17655 and rs751402 genotype frequencies are presented in Table 3. The distribution of rs17655 genotypes in the control group deviated from HWE (P for HWE = 0.02), while that of rs751402 genotypes did not (P for HWE = 0.47). A chi-square test revealed a significant difference in the distribution of *ERCC5* rs751402 genotypes between gastric cancer patients and controls (chi-square = 7.79, P = 0.02), but no such difference was observed in relation to rs17655.

Using unconditional multiple logistic regression analysis, we found that the AA genotype of rs751402 significantly increased gastric cancer risk compared to the GG genotype (OR = 2.61, 95%CI = 1.23-5.49; P = 0.005; Table 4). Moreover, we found that the AA genotype correlated with elevated risk of gastric cancer compared to the GG+AG genotype under a recessive model (OR = 2.21, 95%CI = 1.11-4.39; P = 0.01). However, no significant association was established between rs17655 and gastric cancer risk.

Table 2. Lifestyle and clinical characteristics of gastric cancer patients and control subjects.

Variable	Patients	%	Controls	%	Chi-square	P value	OR(95%CI)	P value
Age, years								
<60	52	36.62	155	43.43			1.0 (Reference)	-
≥60	90	63.38	119	56.57	14.89	<0.001	2.25 (1.46-3.50)	<0.001
Gender								
Female	54	38.03	128	46.72			1.0 (Reference)	-
Male	88	61.97	146	53.28	2.87	0.09	1.43 (0.93-2.21)	0.09
Tobacco smoking								
No	53	37.32	139	50.73			1.0 (Reference)	-
Yes	89	62.68	135	49.27	6.76	0.01	1.73 (1.12-2.68)	0.01
Alcohol drinking								
No	81	57.04	204	74.45			1.0 (Reference)	-
Yes	61	42.96	70	25.55	13.14	<0.001	2.19 (1.40-3.45)	<0.001
Helicobacter pylori infection								
No	55	38.73	157	57.30			1.0 (Reference)	-
Yes	87	61.27	117	42.70	12.90	<0.001	2.12 (1.38-3.28)	<0.001
Clinical stage								
I-II	73	51.41						
III-IV	69	48.59						

OR = odds ratio; CI = confidence interval.

Table 3. Genotype distributions of *ERCC5*rs17655 and rs751402 gene polymorphisms amonggastric cancer patients and control subjects.

SNP	Patients	%	Controls	%	Chi-square	P	P (HWE)
							Controls
rs17655							
CC	56	39.44	118	43.07			
CG	76	53.52	144	52.55			
GG	10	7.04	12	4.38	1.56	0.46	0.02
rs751402							
GG	47	33.10	117	42.70			
AG	73	51.41	136	49.64			
AA	22	15.49	21	7.66	7.79	0.02	0.47

SNP = single nucleotide polymorphism; HWE = Hardy-Weinberg equilibrium.

Table 4. Association between *ERCC5*rs17655 and rs751402 gene polymorphisms and risk of gastric cancer.

SNP	Cases	%	Controls	%	OR (95%CI)[1]	P value
rs17655						
Co-dominant						
CC	56	39.44	118	43.07	1.0 (Ref.)	-
CG	76	53.52	144	52.55	1.11 (0.71-1.74)	0.62
GG	10	7.04	12	4.38	1.76 (0.64-4.73)	0.21
Dominant						
CC	56	39.44	118	43.07	1.0 (Ref.)	-
CG+GG	86	60.56	156	56.93	1.16 (0.75-1.80)	0.48
Recessive						
CC+CG	132	92.96	262	95.62	1.0 (Ref.)	-
GG	10	7.04	12	4.38	1.65 (0.62-4.29)	0.25
rs751402						
Co-dominant						
GG	47	33.1	117	42.7	1.0 (Ref.)	-
AG	73	51.41	136	49.64	1.34 (0.84-2.13)	0.2
AA	22	15.49	21	7.66	2.61 (1.23-5.49)	0.005
Dominant						
GG	47	33.1	117	42.7	1.0 (Ref.)	-
AG+AA	95	66.9	157	57.3	1.51 (0.97-2.36)	0.06
Recessive						
GG+AG	120	84.51	253	92.34	1.0 (Ref.)	-
AA	22	15.49	21	7.66	2.21 (1.11-4.39)	0.01

[1]Adjusted for age, gender, tobacco smoking, alcohol drinking, and *Helicobacterpylori* infection. SNP = single nucleotide polymorphism; OR = odds ratio; CI = confidence interval; Ref. = reference.

DISCUSSION

In this study, we investigated the relationship between the *ERCC5* rs17655 and rs751402 polymorphisms and development of gastric cancer. We found a certain genotype of rs751402 to increase gastric cancer risk significantly in comparison to the wild-type sequence. Previous studies have investigated associations between *ERCC5* gene polymorphisms and risk of various cancers (Luo et al., 2013; Liang et al., 2014; Lu et al., 2014; Na et al., 2015; Zeng et al., 2015). Luo et al. (2013) conducted a case-control study to assess the influence of several single nucleotide polymorphisms of DNA repair genes on glioma risk, finding that the *ERCC5*Asp1558Hisvariation is connected to susceptibility to this cancer. In a meta-analysis involving nine studies, Liang et al. (2014) found that *ERCC5* rs17655 may not contribute to genetic susceptibility to lung cancer. However, Lu et al. (2014) carried out a case-control investigation in a Chinese population, from which they concluded that certain variants of this same polymorphism are associated with increased risk of laryngeal cancer. In a separate Chinese case-control study, Na et al. (2015) failed to connect rs751402 with breast cancer risk, while a nine-study meta-analysis conducted by Zeng et al. (2015) suggested that the *ERCC5* rs17655 polymorphism might contribute to colorectal cancer genetic susceptibility. Two reports have tested the association between *ERCC5* gene polymorphisms and gastric cancer development, but with inconclusive results (Hussain et al., 2009; Duan et al., 2012). Hussain et al. (2009) examined an American population, in which they found that the rs1047768, rs17655, and rs2227869 polymorphisms are associated with gastric cancer. In addition, Duan et al. (2012) reported that *ERCC5* rs751402 and rs2296147 polymorphisms may alter the risk of developing this disease, particularly the diffuse subtypes. In our study, we found that *ERCC5* rs751402 affects gastric cancer risk, but rs17655 does not. There are two limitations tothe present work. First, the participants were recruited from a single city hospital; therefore, this study group might not be particularly representative of other populations in China and other ethnicities worldwide. Second, the sample size of our study was not large, which may have compromised the power of the statistical tests used to discern differences between groups. Therefore, further studies with greater sample sizes are greatly required to validate the results of our investigation. In conclusion, we suggest that the *ERCC5* rs751402 polymorphism is associated with development of gastric cancer, while the rs17655 variant is not. Further work using larger study populations is greatly needed to firmly establish the relationship between *ERCC5* gene polymorphisms and gastric cancer risk.

Conflicts of interest

The authors declare no conflict of interest.

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