RESEARCH ARTICLE

The DNA Repair Gene *ERCC6* rs1917799 Polymorphism is Associated with Gastric Cancer Risk in Chinese

Jing-Wei Liu, Cai-Yun He, Li-Ping Sun, Qian Xu, Cheng-Zhong Xing*, Yuan Yuan*

Abstract

Objective: Excision repair cross-complementing group 6 (ERCC6) is a major component of the nucleotide excision repair pathway that plays an important role in maintaining genomic stability and integrity. Several recent studies suggested a link of *ERCC6* polymorphisms with susceptibility to various cancers. However, the relation of *ERCC6* polymorphism with gastric cancer (GC) risk remains elusive. In this sex- and age- matched case-control study including 402 GC cases and 804 cancer-free controls, we aimed to investigate the association between a potentially functional polymorphism (rs1917799 T>G) in the *ERCC6* regulatory region and GC risk. Methods: The genotypes of rs1917799 were determined by Sequenom MassARRAY platform and the status of *Helicobacter pylori* infection was detected by enzyme-linked immunosorbent assay. Odd ratios (ORs) and 95% confidential interval (CI) were calculated by logistic regression analysis. Results: Compared with the common TT genotype, the *ERCC6* rs1917799 GG genotype was associated with increased GC risk (adjusted OR=1.46, 95%CI: 1.03-2.08, *P*=0.035). When compared with (GT+TT) genotypes, the GG genotype also demonstrated a statistical association with increased GC risk (adjusted OR=1.38, 95%CI: 1.01-1.89, *P*=0.044). This was also observed for the male subpopulation (GG vs. TT: adjusted OR=1.71, 95%CI: 1.12-2.62, *P*=0.013; G allele vs. T allele: adjusted OR=1.32, 95%CI: 1.07-1.62, *P*=0.009). Genetic effects on increased GC risk tended to be enhanced by *H. pylori* infection, smoking and drinking, but their interaction effects on GC risk did not reach statistical significance. Conclusions: *ERCC6* rs1917799 GG genotype might be associated with increased GC risk in Chinese, especially in males.

Keywords: *ERCC6/CSB* - polymorphism - susceptibility - gastric cancer

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Introduction

Gastric carcinogenesis is a complicated multifactorial process involving host genetics, lifestyle and environmental factors (Lochhead et al., 2008; Correa, 2013). Since the stomach is constantly exposed to both endogenous and exogenous stimulation, damage of gastric cellular DNA seems to be a frequent event (Farinati et al., 2008). The human DNA repair network is therefore essential for the maintenance of genome stability and integrity, thereby preventing gastric cancer (GC) (Laine et al., 2006; Rechkunova et al., 2010). It is reasonable that sequence variations of the genes accounting for major components of human DNA repair system may be implicated in the genetic background of GC (Berwick et al., 2000).

Nucleotide excision repair (NER) is a critical and versatile DNA repair system that eliminates a broad spectrum of structural DNA lesions including ultraviolet-induced cyclobutane pyrimidine dimmers, bulky adducts and DNA cross-links (de Laat et al., 1999). Global-genomic repair and transcription-coupled repair are two main pathways of NER (Friedberg, 2001). The excision repair cross-complementing group 6 (*ERCC6*) gene, alternatively known as Cockayne syndrome complementation group B (CSB), is an essential factor of transcription-coupled NER, which allows RNA polymerase II-blocking lesions to be rapidly removed from the transcribed strand of active genes (Troelstra et al., 1992). Defects of *ERCC6* gene was firstly found to be the cause of Cockayne syndrome (Boraz, 1991). Subsequently, disruption of this gene was related to the development of age-related macular degeneration (Tuo et al., 2006). Quite recently, genetic variations of *ERCC6* have been linked to the susceptibility to various cancers, including lung cancer (Lin et al., 2008; Ma et al., 2009), breast cancer (Mechanic et al., 2006; Rajaraman et al., 2008), prostate cancer (Hooker et al., 2008), bladder cancer (Chen et al., 2007; Chang et al., 2009) and colorectal cancer (Berndt et al., 2006; Huang et al., 2006). However, the relation of *ERCC6* polymorphism with gastric cancer risk is still unclear, which deserves to be further clarified.

In the present study, we focused on single nucleotide polymorphism (SNP) in the regulatory region of *ERCC6* gene (from -5000bp to +200bp, +1 represents the site

*Tumor Etiology and Screening Department of Cancer Institute and General Surgery, the First Affiliated Hospital of China Medical University, and Key Laboratory of Cancer Etiology and Prevention (China Medical University), Liaoning Provincial Education Department, Shenyang, China *For correspondence: xcz1966@126.com, yyuan@mail.cmu.edu.cn*

where transcription starts). Among the currently-known SNPs in this region, rs1917799 was indicated as a tagSNP to present another polymorphism rs11594945. Furthermore, rs1917799 polymorphism was predicted to be a transcription factor binding site of RAR alpha1, which demonstrated potential effect on the regulation of *ERCC6* transcription (Yuan et al., 2006). Moreover, this SNP is frequently expressed in various ethnicities. Therefore, we investigated the association of rs1917799 polymorphism with GC risk in the current study. Apart from the genetic effect, its interaction with several potential environmental risk factors was also evaluated, including the status of *H pylori* infection and consumption of smoking and alcohol drinking.

Materials and Methods

Study design and study population

In this study, a total of 1854 individuals consisting of 478 GC cases and 1376 cancer-free controls were retrospectively recruited from a multicentre research in Liaoning Province, Northeast China between 1997 and 2011. All the enrolled subjects were diagnosed based on the gastroscopic and histopathological examinations. Patients with a history of other malignant tumors were excluded from our study. The eligible controls were the subjects with normal stomach or only gastritis. Other information of the enrolled subjects was extracted from registered documents, including sex, age, status of smoking and alcohol drinking consumption. For association analysis in this study, cancer-free controls were frequency-matched to GC cases by sex and age (±5 years). Accordingly, 402 GC cases and 804 cancer-free controls were included for final analysis. In addition, we classified the GC cases into intestinal-type and diffuse-types based on the Lauren's classification (Lauren, 1965). Subjects who smoked currently or quit smoking for less than one year were defined as smokers; those who had never smoked or smoked for no more than one year were considered as nonsmokers. Individuals that drank at least once a week for more than one year were defined as drinkers while the rest were thought to be nondrinkers.

Ethics approval statement

The design of this study was approved by the Human Ethics Committee of China Medical University (Shenyang, China) before the outset of the research. Each individual involved in the study provided us with the written informed consents during epidemiological investigation.

*The selection of **ERCC6** rs1917799 polymorphism*

Genotype data of SNPs in *ERCC6* regulatory region (from -5000bp to +200bp, +1 represents the site where transcription starts) of CHB (Chinese Han Beijing) population were extracted from International HapMap Project (Release #27, Phase1, 2 & 3, http://www.hapmap.org). A total of nine SNPs were found in this region (Figure 1), while only three SNPs (i.e. rs4253002, rs1917799 and rs11594945) showed common genotype frequencies (minor allele frequency (MAF) >0.05) as well as

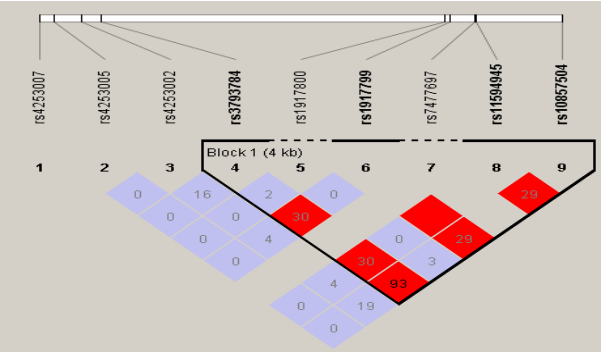

Figure 1. Plot of Information of Linkage-disequilibrium (r^2) among Polymorphisms of *ERCC6* Regulatory Region Based on CHB HapMap Population

genotyping rates >75%. TagSNP for three common SNPs was selected based on pairwise linkage disequilibrium (LD) algorithm using Haploview software (Version 4.2). Accordingly, the rs1917799 was indicated as a tagSNP to present rs11594945 for a complete linkage disequilibrium with each other (r^2=1). By further predicting their genetic functions using FASTSNP software (Yuan et al., 2006) (available online: http://fastsnp.ibms.sinica.edu.tw/pages/input_CandidateGeneSearch.jsp), rs1917799 was predicted as a transcription factor binding site of RAR alpha1, which demonstrated a potential effect on the regulation of its gene's transcription; while rs4253002 showed no predicted effect on the regulation of this gene's transcription. Finally, rs1917799 polymorphism was selected to investigate whether the polymorphism in *ERCC6* regulatory region is associated with susceptibility to GC.

*Genotyping of **ERCC6** rs1917799 polymorphism*

Genomic DNA of the blood samples from included subjects was extracted using routine phenol-chloroform method and then diluted to working concentration (50 ng/μl) for *ERCC6* rs1917799 genotyping. All of the samples were placed randomly on the 384-well plates and blinded for the status of the disease. The design of the assay and *ERCC6* rs1917799 genotyping were carried out CapitalBio (Beijing, China) using Sequenom MassARRAY platform (Sequenom, San Diego, California, USA) based on the manufacturer's directions. To evaluate the quality of the genotyping, 50 samples were genotyped repeatedly and the results were 100% consistent.

***H pylori** serology examination*

The test of *H pylori* serology was performed to check the status of *H pylori* infection using enzyme-linked immunosorbent assay (ELISA, *H pylori*-IgG ELISA kit, BIOHIT Plc, Helsinki, Finland), as described previously (Gong et al., 2010). Briefly, approximately 5 ml fasting venous blood was obtained from each individual and the serum sample was collected after 10 min centrifugation at a speed of 3500×g. *H pylori*-immunoglobin (Ig) G concentrations of the serum sample were detected by ELISA kit (BIOHIT Plc, Helsinki, Finland) according to the manufacturer's protocol. A numerical reading exceeding 34 enzyme immune-units (EIU) was considered to be *H pylori* infection positive.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) for *ERCC6* rs1917799 was firstly evaluated among cancer-free controls. The average age was expressed as mean±SD and the age difference between cases and controls groups was assessed using Student's t test. Pearson's χ^2 test was applied to evaluate the differences of categorical variables including gender, *H pylori* infection, smoking status and drinking status. The adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) of the relation between *ERCC6* rs1917799 polymorphism and GC risk were calculated by multivariate logistic regression with adjustments for gender, age and *H pylori* infection status. Likelihood ratio test was performed to assess the interaction effects of genotype and potential environmental risk factors on the risk of GC by comparing the model only involving main effects of gender, age, environment factors and genotype with the full model also containing the interaction term of genotype with environment factor. All of the statistical analyses above were carried out by using SPSS 13.0 software (SPSS, Chicago, IL, USA). $P<0.05$ for all two-sided tests was regarded as statistically significant. The statistical power for this case-control genetic association study was assessed using PGA software (http://dceg.cancer.gov/tools/analysis/pga).

Results

Baseline characteristics of the study population and genotype frequency

The baseline characteristics of the study population were presented in Table 1. Before matching the cancer-free controls to GC cases, there were statistical differences in the distribution of age and sex between the two groups (both $P<0.001$). No such differences existed after the matching for age and sex ($P=0.979$ for age, $P=1.000$ for gender). Therefore, the strength of the association of *ERCC6* rs1917799 polymorphism with GC risk was assessed in the frequency-matched 402 GC cases and 804 controls. *H pylori* infection positive rate in GC cases (53.2%) was higher than that in cancer-free controls (41.4%), which demonstrated significant difference ($P<0.001$).

The distribution of genotype and allele frequencies of *ERCC6* rs1917799 in matched samples was showed in Table 2. The separate frequencies of common TT, heterozygous GT and rare GG genotypes of rs1917799 were 35.8%, 48.5% and 15.7% in the control group and 32.1%, 48.0% and 19.9% in case group. The genotypes among the controls were in agreement with HWE ($P=0.751$). A statistical power of 0.991 was evaluated by PGA software with settings of $\alpha= 0.05$ and OR=2.

*Association between **ERCC6** rs1917799 genotypes and GC risk*

The association of rs1917799 polymorphism with the risk of overall GC risk was presented in Table 2. In the total population, compared with common TT genotype, the rare GG genotype was observed to be associated with increased GC risk (adjusted OR=1.46, 95%CI: 1.03-2.08, $P=0.035$) while no association was found between GT genotype and GC risk (adjusted OR=1.09, 95%CI: 0.83-1.43, $P=0.549$). When compared with the GT and TT genotypes, the rare GG genotype still demonstrated a significant association with increased GC risk (adjusted OR=1.38, 95%CI: 1.01-1.89, $P=0.044$). Moreover, the G allele was marginally associated with increased GC risk compared with T allele (adjusted OR=1.18, 95%CI: 1.00-1.41, $P=0.055$).

Genetic effect of rs1917799 polymorphism on the risk of GC was also assessed in subpopulations according to sex and age, and also in histological subtype for GC case based on the Lauran's classification. In the stratified analysis of histology (Table 3), the rare GG genotype was marginally associated with increased risk of intestinal-type GC either compared with TT or (GT+TT) genotypes (for GG vs. TT: adjusted OR=1.58, 95%CI: 0.93-2.68, $P=0.093$; for GG vs. GT+TT: adjusted OR=1.52, 95%CI: 0.95-2.41, $P=0.080$) although these did not reach statistical significance. In the stratified analysis of sex (Table 3), statistically significant association of rs1917799 GG genotype with increased GC risk was observed in the male subgroup. Compared with the common TT genotype, GG genotype was associated with increased GC risk (adjusted OR=1.71, 95%CI: 1.12-2.62, $P=0.013$). The most significant association was observed in the allele analysis (G allele vs. T allele: adjusted OR=1.32, 95%CI: 1.07-1.62, $P=0.009$). As for the stratified analysis of age,

Table 1. Baseline Characteristic of Study Population for *ERCC6* rs1917799 Polymorphism

	GC cases	Cancer-free controls	*p* value
Total sample(n)	478	1376	
Age(mean±SD, year)	59.02±11.09	54.00±9.49	<0.001
Range	26-87	16-85	
Gender			<0.001
Male	325 (68.0%)	737 (53.6%)	
Female	153 (32.0%)	639 (46.4%)	
Matched sample(n)	402	804	
Age(mean±SD, year)	56.41±8.72	56.39±8.64	0.979
Range	30-84	28-85	
Gender			1.000
Male	273 (67.9%)	546 (67.9%)	
Female	129 (32.1%)	258 (32.1%)	
Lauren's classification*			
Intestinal-type GC	130 (43.9%)	-	
Diffuse-type GC	166 (56.1%)	-	
H pylori infection			<0.001
Positive	214 (53.2%)	333 (41.4%)	
Negative	188 (46.8%)	471 (58.6%)	

*Some of the GC cases failed to be classified into either group of intestinal-type or diffuse-type GC; Abbreviation: GC, gastric cancer

Table 2. Association between *ERCC6* rs1917799 Polymorphism and Gastric Cancer Risk

Variables	Cancer-free controls	HWE *P*	GC		
	n (%)		n (%)	Adj. OR (95%CI)*	*p* value
TT	288 (35.8)	0.751	129 (32.1)	1 (ref)	
GT	390 (48.5)		193 (48.0)	1.09 (0.83, 1.43)	0.549
GG	126 (15.7)		80 (19.9)	**1.46 (1.03, 2.08)**	**0.035**
GG:(GT+TT)				**1.38 (1.01, 1.89)**	**0.044**
T allele	966 (60.1)		451 (56.1)	1 (ref)	
G allele	642 (39.9)		353 (43.9)	1.18 (1.00, 1.41)	0.055

*Abbreviations: GC, gastric cancer; HWE, Hardy-Weinberg equilibrium

Table 3. Stratification Analysis of Association between *ERCC6* rs1917799 Polymorphism and Gastric Cancer Risk

		Variables	GC cases	Controls	Adj. OR (95%CI)*	*p*
Age	≥60	TT	43	93	1(ref)	
		GT	69	152	0.95(0.60, 1.51)	0.821
		GG	29	42	1.54(0.84, 2.81)	0.162
		GG:(GT+TT)			1.57(0.93, 2.67)	0.092
		T allele			1(ref)	
		G allele			1.18(0.89, 1.58)	0.255
	<60	TT	86	195	1(ref)	
		GT	124	238	1.16(0.83, 1.63)	0.386
		GG	51	84	1.45(0.93, 2.24)	0.099
		GG:(GT+TT)			1.28(0.87, 1.89)	0.214
		T allele			1(ref)	
		G allele			1.19(0.96, 1.47)	0.122
Gender	Male					
		TT	78	201	1(ref)	
		GT	137	256	1.37(0.98, 1.91)	0.069
		GG	58	89	**1.71(1.12, 2.62)**	**0.013**
		GG:(GT+TT)			1.43(0.99, 2.07)	0.060
		T allele			1(ref)	
		G allele			**1.32(1.07, 1.62)**	**0.009**
	Female					
		TT	51	87	1(ref)	
		GT	56	134	0.69(0.43, 1.10)	0.118
		GG	22	37	1.02(0.54, 1.94)	0.942
		GG:(GT+TT)			1.26(0.71, 2.26)	0.430
		T allele			1(ref)	
		G allele			0.94(0.69, 1.27)	0.668
Type	Intestinal-type					
		TT	41	288	1(ref)	
		GT	61	390	1.07(0.70, 1.65)	0.743
		GG	28	126	1.58(0.93, 2.68)	0.093
		GG:(GT+TT)			1.52(0.95, 2.41)	0.080
		T allele			1(ref)	
		G allele			1.23(0.94, 1.60)	0.131
	Diffuse-type					
		TT	57	288	1(ref)	
		GT	83	390	1.07(0.73, 1.55)	0.742
		GG	26	126	1.09(0.65, 1.82)	0.753
		GG:(GT+TT)			1.04(0.66, 1.66)	0.863
		T allele			1(ref)	
		G allele			1.04(0.82, 1.33)	0.727

*OR and 95%CI were calculated by logistic regression with adjustments of other factors; Abbreviations: GC, gastric cancer; ref, reference

the GG genotype tended to be associated with increased GC risk either in the age≥ 60 years or age< 60 years subgroups (Table 3).

*Interaction analysis between **ERCC6** rs1917799 genotypes and environmental risk factors*

The subjects with available information of *H pylori* infection, smoking and drinking were included for interaction analysis. Because we observed a positive association of GG genotype with GC risk, the (GT+TT) carriers without *H pylori* infection or consumption of smoking and drinking were regarded as reference for each interaction analysis. The results of interaction effects on the risk of overall and each subtype GC were summarized in Table 4. In the overall analysis, we found that the risk of GC was enhanced by *H pylori* positive, ever-smoking and alcohol drinking respectively for individuals carrying GG genotype, with corresponding adjusted ORs of 1.88 (95%CI: 1.25-2.85), 2.92 (95%CI: 1.63-5.22) and 2.43 (95%CI: 1.19-4.97). In the intestinal subtype analysis, GC risk for subjects carrying GG genotype was also enhanced by *H pylori* infection (OR=3.04, 95%CI: 1.69-5.48) but not by smoking or drinking. As for the risk of diffuse subtype GC, all associations did not reach statistical significant level. We did not found any interaction effect of rs1917799 genotypes with the above described environment factors on the risk of GC in a multiplicative interactive model (all *P* values for interaction analyses were larger than 0.05).

Discussion

To our knowledge, this is the first study to evaluate the association of *ERCC6* polymorphism with the risk of GC. The findings of this gender- and age- matched study demonstrated an association of rs1917799 polymorphism in *ERCC6* regulatory region with the risk of GC. This association was also evident in subpopulation of males. Additionally, its genetic effect on increased GC risk seemed to be enhanced in the context of *H pylori* infection, smoking and alcohol drinking, although their interaction

Table 4. Interaction between *ERCC6* Genotypes and Environment Factors

		All-type GC		Intestinal-type GC		Diffuse-type GC	
		TT+GT	GG	TT+GT	GG	TT+GT	GG
H pylori infection							
Negative	Cases/Controls	178/683	49/137	53/683	15/137	79/683	20/137
	Adjusted OR(95%CI)*	1(ref)	1.31(0.90, 1.91)	1(ref)	1.28(0.69, 2.39)	1(ref)	1.21(0.72, 2.06)
Positive	Cases/Controls	208/462	40/90	75/462	19/90	88/462	10/90
	Adjusted OR(95%CI)*	1.70(1.34, 2.16)	1.88(1.25, 2.85)	2.02(1.38, 2.96)	3.04(1.69, 5.48)	1.63(1.17, 2.26)	1.01(0.50, 2.03)
		P for interaction=0.551**		*P* for interaction=0.710**		*P* for interaction=0.132**	
Smoking							
Nonsmoker	Cases/Controls	113/598	25/120	47/598	8/120	49/598	12/120
	Adjusted OR(95%CI)*	1(ref)	1.11(0.68, 1.81)	1(ref)	0.84(0.38, 1.88)	1(ref)	1.26(0.64, 2.46)
Smoker	Cases/Controls	100/252	24/49	34/252	7/49	36/252	4/49
	Adjusted OR(95%CI)*	2.15(1.49, 3.12)	2.92(1.63, 5.22)	1.44(0.83, 2.48)	1.86(0.74, 4.63)	2.03(1.17, 3.52)	1.19(0.39, 3.64)
		P for interaction=0.601**		*P* for interaction=0.483**		*P* for interaction=0.244**	
Alcohol drinking							
Nondrinker	Cases/Controls	114/668	24/138	51/668	10/138	52/668	10/138
	Adjusted OR(95%CI)*	1(ref)	1.08(0.66, 1.77)	1(ref)	1.01(0.49, 2.09)	1(ref)	0.98(0.48, 1.99)
Drinker	Cases/Controls	69/182	14/31	30/182	4/31	30/182	6/31
	Adjusted OR(95%CI)*	2.10(1.42, 3.11)	2.43(1.19, 4.97)	1.68(0.97, 2.90)	1.32(0.43, 4.06)	2.36(1.35, 4.13)	2.58(0.97, 6.09)
		P for interaction=0.871**		*P* for interaction=0.710**		*P* for interaction=0.857**	

*OR and 95%CI were calculated by logistic regression with adjustment; **Interaction effects of *ERCC6* genotypes and environmental factors on GC risk were evaluated by Likelihood ratio test using a full model

effects didn't reach statistical significance.

ERCC6 gene is located at chromosome 10q11.23 of human, encoding a 168 kDa protein (Fousteri et al., 2008). The *ERCC6* (CSB) protein has been identified as an indispensable component of transcription coupled repair pathway of NER which is an effective and versatile DNA repair system keeping human genome stability and integrity (Costa et al., 2003; Stevnsner et al., 2008). This protein promotes the production of functional multiprotein complex at the DNA repair site to verify proper synthesis of RNA through interacting with several other essential proteins (Lagerwerf et al., 2011; Sugasawa, 2011). The *ERCC6* protein is also characterized by seven typical ATPase domains and demonstrates DNA-dependent ATPase activity (van Hoffen et al., 2003). Mutations of *ERCC6* gene are frequently observed in Cockayne syndrome which is a hereditary autosomal recessive disease (Licht et al., 2003). *ERCC6* genetic variation is also linked to age-related macular degeneration which can result in irreversible visual loss. Recently, several studies have reported certain association of *ERCC6* polymorphisms with susceptibilities to different cancers. In animal models, mice with disrupted *ERCC6* gene were more likely to develop skin cancer when constantly exposed to UV radiation (van der Horst et al., 1997). Sequence variations of *ERCC6* may change transcription and expression process whereby the role of *ERCC6* protein in the TCNER pathway might be altered. In that way, the observed association of *ERCC6* gene polymorphism with increased cancer risk could be partially explained.

Sequence variation especially the polymorphic site changing the binding activity of certain transcription factor in promoter/regulatory region hold great promise in altering the regulation of the gene's transcription and thereby modulating cancer risk (Trzyna et al., 2012). Driven by such a hypothesis, rs1917799 T>G polymorphism, located in regulatory region of *ERCC6* gene and predicted as a transcription factor binding site of RAR alpha1 (FASTSNP, http://fastsnp.ibms.sinica.edu.tw/pages/input_CandidateGeneSearch.jsp), was selected to investigated in the current study. The variant GG genotype of rs1917799 was found to confer a 1.46-fold overall risk of GC development when compared with the common TT homozygote. Based on the predicted function effects, the change from T to G allele would loss the binding ability of RAR alpha1 which may partially explain the association of variant G with an increased GC risk. However, further studies are still required to confirm this predicted biological significance. By reviewing the currently available literatures on *ERCC6* polymorphisms, we noted that associations of another frequently-reported SNP rs2228528 with different cancers have been described including oral cancer (Chiu et al., 2008), lung cancer (Ma et al., 2009) and bladder cancer (Chang et al., 2009). The rs2228528 polymorphism located in the exon 5 of *ERCC6* gene is in highly linkage disequilibrium with the currently-investigated SNP rs1917799 (r^2=0.95, derived from CHB genotype data of HapMap Project). Consistent positive associations of either rs1917799 or rs2228528 polymorphism with cancer risks may indirectly provide certain clues that link *ERCC6* genetic variations with cancer development. Thus, rs1917799 polymorphism of *ERCC6* may be associated with increased GC risk.

Different histological GC is thought to bear its distinct pathogenic mechanism that involves distinguishing genetic components (Vauhkonen et al., 2006). Stratification analysis of the histology of GC was therefore performed to explore whether the association strength of rs1917799 with GC risk differed in intestinal and diffuse subtypes. In rs1917799 GG genotype subjects, we observed a 1.58-fold increased risk for intestinal-type GC while no identifiable risk (OR=1.09) for diffuse-type GC, which indicated a tendency of association between this polymorphism with intestinal GC risk. However, only large-scale study in future could draw a more reliable conclusion on this difference. Apart from histology, stratification analysis of gender presented a more evident association of rs1917799 polymorphism with increased GC risk in males. In general, men are more vulnerable to GC and their mortality rate of GC exceed that in women (Jemal et al., 2011). Higher exposure rate to environmental risk factors such as smoking and drinking and unhealthy living habits in males may also add additional risk for GC development (Brenner et al., 2009). These factors together may, at least in part, explain why a more perceptible association of rs1917799 polymorphism with GC risk in males was observed.

Stomach is continuously under stimulation from various endogenous and exogenous factors, leading to a dynamic balance between damage and repair (Zabaleta, 2012). Several environmental factors such as *H pylori* infection and consumption of smoking and alcohol have been identified or suspected to add the risk of cancer (Compare et al., 2010; Sankari et al., 2012). *H pylori* infection, smoking and drinking may aggravate the burden of DNA repair thereby add additional risk for GC development, especially in subjects with insufficient DNA repairing capacity. In the present study, no significant association was observed of *ERCC6* rs1917799 with GC risk in different environmental factor subgroup by stratification analysis. Besides, no statistically significant interaction effect between rs1917799 and environment risk factors was observed in overall GC group or in intestinal- and diffuse-type GC subgroups, which indicating such interactive effect between *ERCC6* rs1917799 polymorphism and environmental factors may not exist in our study sample. However, we observed that the risks of GC development in individuals carrying GG genotypes were enhanced by environment risk factors including *H pylori* positive, ever-smoking and alcohol drinking. Further interaction analysis evaluating larger population is still in need to ensure the results.

We were aware that several limitations existed in our study. One major limitation is that our study sample size is relatively insufficient especially for stratification and interaction analyses. Thus, the results from stratified analysis and interaction analyses were only considered as exploratory screening. Moreover, molecular evidences about the modulation of DNA transcription and mRNA expression are warranted to support the result of our study.

In conclusion, rs1917799 polymorphism in *ERCC6* regulatory region might be associated with increased GC risk in Chinese, especially in males. No significant

interaction effect was observed between *ERCC6* rs1917799 polymorphism and *H pylori* infection, smoking and drinking on the risk of GC. Functional studies and future large-scale study particularly in other ethnic populations are still needed to validate our findings.

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