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Original Article

IL-4/IL-4R and *IL-6/IL-6R* genetic variations and gastric cancer risk in the Chinese population

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Abstract: Objective: The IL-4/IL-4R and IL-6/IL-6R signaling pathways are involved in immune response and play roles in gastric carcinogenesis. To investigate the association between *IL-4/IL-4R* and *IL-6/IL-6R* genetic variations and gastric cancer risk, and their prognostic values, we performed a case-control study. The genotypes of the genetic variations were detected using a Mass-array platform. The *Helicobacter pylori* infection status was determined using a commercial *H. pylori* immunogold testing kit. We found that the *IL-6* rs1800796 G allele was associated with an increased risk of gastric cancer (GG *vs.* CC: $OR_{adjusted}$ = 2.20, 95% CI = 1.33-3.63; GG/CG *vs.* CC: $OR_{adjusted}$ = 1.41, 95% CI = 1.09-1.82). The stratified analysis showed that rs1800796 G allele carriers (GG/CG) were associated with an increased risk of gastric cancer in the following subgroups: age >64 years old ($OR_{adjusted}$ = 1.67, 95% CI = 1.17-2.39), female ($OR_{adjusted}$ = 1.82, 95% CI = 1.09-3.05), positive for *H. pylori* infection ($OR_{adjusted}$ = 1.54, 95% CI = 1.07-2.22), non-cardiac gastric cancer ($OR_{adjusted}$ = 1.53, 95% CI = 1.15-2.04), stage T3-T4 tumor ($OR_{adjusted}$ = 1.41, 95% CI = 1.06-1.88), and gastric cancer with median to high differentiation ($OR_{adjusted}$ = 1.45, 95% CI = 1.08-1.96). None of the genetic variations were associated with overall survival. In short, we concluded that the *IL-6* rs1800796 GG genotype is a risk factor for gastric cancer and that rs1800796 G allele carriers have an increased risk of gastric cancer; this association was stronger in individuals that were >64 years old, female, or positive for *H. pylori* infection. None of the genetic variations were associated with gastric cancer prognosis.

Keywords: *IL-4*, *IL-4R*, *IL-6*, *IL-6R*, genetic variation, gastric cancer, associations

Introduction

Gastric cancer is the fifth-most common type of cancer and the second-most prevalent cause of cancer-related deaths worldwide [1]. More than one-half of gastric cancer cases worldwide are diagnosed in eastern Asia, predominantly in China [2]. Gastric cancer is a complex and progressive disease; complex genetic and environmental interactions contribute to its initiation and progression [3]. Environmental factors, including *Helicobacter pylori* infection, excessive salt or alcohol intake, smoking, and genetic variations [4] are all risk factors for the development of gastric cancer.

In 1994, the World Health Organization and the International Agency for Research on Cancer identified *H. pylori* as a class I carcinogen. The infection rate of *H. pylori* is approximately 40-50% of the global human population, and studies have shown that people with *H. pylori* infection are five times more likely to develop gastric cancer than people without *H. pylori* infection [5, 6]. *H. pylori* colonization can cause chronic inflammation, which significantly increases the risk of developing duodenal and gastric ulcers and gastric cancer. Epidemiologic studies have suggested that the interaction between the host genetic background and *H. pylori* contributes to the development of gastric cancer. *IL1B*, a proinflammatory factor that mediates the inflammatory response and strongly inhibits gastric acid secretion [7], is upregulated in response to *H. pylori* infection. In addition, we have shown that genetic variations in *IL-1B* associate with the risk of gastric cancer [8, 9]. The host immune response that is triggered by *H. pylori*

could be balanced by an anti-inflammatory response mediated by T helper 2 (Th2) cytokines, such as IL-10 and IL-4. We have shown that a shift in the Th1/Th2 balance can predict the progression of gastric disease caused by *H. pylori* [10]. Although it has been hypothesized that genetic variations in *IL-4/IL-4R* associate with gastric cancer risk, the results of epidemiologic studies have been inconclusive and have shown differences among different ethnic backgrounds [11-13]. IL-6 acts as both a pro- and anti-inflammatory cytokine [14] and regulates the immune response. It has also been shown that IL-6 may facilitate carcinogenesis through several mechanisms [15]. Epidemiologic studies have investigated the association of *IL-6/IL-6R* genetic variants with the risk [13, 16-20] and prognosis [21] of gastric cancer, but the conclusions have been inconsistent [20, 22-24]. Thus, we performed a case-control study to assess the association of *IL-4/IL-4R* and *IL-6/IL-6R* genetic variations with the risk and prognosis (determined by the number of overall survival (OS) days of gastric cancer.

Materials and methods

Study subjects

This case-control study enrolled 479 gastric cancer patients and 483 age- and sex-matched healthy controls. All patients were histologically diagnosed to have gastric cancer, and the controls were individuals who came to the hospital for routine physical examinations. Data on the demographic features of the healthy controls were collected via a questionnaire, and data on the clinical features of the cancer patients were collected from their medical records. The clinical stages of the cancers were classified according to the American Joint Commission for Cancer Staging Manual, 6th edition. The survival status of patients were obtained by on-site interviews, direct calling, or reviews of medical charts. The Institutional Review Board of the Nanjing First Hospital approved this protocol, and written informed consent was obtained from all of the participants.

DNA extraction and genotyping

First, we selected the *IL-4/IL-4R* and *IL-6/IL6R* genetic variations to evaluate by reviewing the literature on *IL-4/IL-4R* and *IL-6/IL6R* genetic variants and their associations with gastric cancer risk. For the genetic variations that we selected, we retrieved related information from the National Center for Biotechnology Information dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP). Then, we selected the genetic variations based on the following the criteria: (1) the minor allele frequency (MAF) was not <5% in the Han Chinese population; (2) the variation was positioned in an exon, promoter region, 5'untranslated region (UTR), or 3'UTR; and (3) the variation has been associated with cancer risk. Finally, we chose six *IL-4/IL-4R* genetic variations and four *IL-6/IL-6R* genetic variations to study further (Table S1).

DNA extraction and genotyping were performed as previously described [25]. In brief, DNA was extracted using the GoldMag-Mini Whole Blood Genomic DNA Purification Kit according to the manufacture's protocol (GoldMag Co. Ltd. Xi'an, China). DNA purity was evaluated by spectrophotometry (DU530UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA), and genotyping was performed with Sequenom MassARRAY RS1000 according to the standard protocol. We used MassARRAY Assay Design version 3.1.2.2 (Sequenom Inc., San Diego, CA) to design PCR and extension primers for the genetic variations. After PCR, the amplified DNA products were cleaned using EXOSAP (Sequenom), extended by IPLEX chemistry, desalted using Clean Resin (Sequenom), and spotted onto Spectrochip matrix chips using a nanodispenser (Samsung). The chips were run in duplicate on a Sequenom MassArray MALDI-TOF MassArray system. To confirm the genotyping results, we randomly selected 15% of the samples for re-genotyping, and the results showed a concordance rate of 100%.

H. pylori serum assays

To determine whether the study participants had *H. pylori* infections, a commercial *H. pylori* immunogold testing kit (Kangmei Tianhong Biotech Co., Ltd, Beijing, China) was used to test for *H. pylori* antibodies; this test had a sensitivity of 98.29% and a specificity of 98.51% for the detection of *H. pylori* infection in the Chinese population.

Statistical analysis

The differences in the demographic features of the two groups were assessed by the *t* test or the chi-square (χ^2) test. Hardy-Weinberg equilibrium (HWE) in the control group was tested

using a goodness of fit χ^2 test. The odds ratios (ORs) and 95% confidence intervals (CIs) for the association of the genetic variations with the risk of gastric cancer were calculated using a logistic regression based on SAS software (Version 9.1; SAS Institute, Cary, NC, USA). A sub-group analysis based on clinical and pathological characteristics was conducted to check if there was a significant association between the genetic variation and gastric cancer risk. Survival curves were assessed by Kaplan-Meier analyses. The association between the survival time and the genetic variation was estimated using the log-rank test. Hazard ratios (HRs), which were used to determine the prognostic values of the genetic variations for patient survival, were calculated using univariate Cox regression models or multivariate Cox regression models if the genetic variation was significantly associated with gastric cancer risk. The data was calculated using SPSS 11.0 software (SPSS, Chicago, IL, USA). A two-sided *p*-value <0.05 was considered statistically significant.

Results

Characteristics of the study population

The demographic and exposure data of all the participants are summarized in Table S2. There were no differences between the two groups with respect to age and gender (age: $P = 0.748$, gender: $P = 0.881$). The frequencies of *H. pylori* infection, cigarette smoking, and alcohol consumption were higher in the study patients than in the controls (*H. pylori*: $P = 0.039$, cigarette smoking: $P<0.001$, alcohol consumption: $P<0.001$). The observed frequencies of all of the tested genotypes in the controls did not deviate from HWE (Table S1). The distributions of the genetic variations in the case patients and the controls are presented in **Table 1**.

Associations between genetic variations and gastric cancer risk

There was a significant difference in the distribution of the rs1800796 genotype between the cancer group and the control group. Logistic regression revealed that the rs1800796 GG and GG/CG genotypes were associated with increased gastric cancer risk (GG *vs.* CC: adjusted OR = 2.20, 95% CI = 1.33-3.63, $P = 0.002$; GG/CG *vs.* CC: adjusted OR = 0.009, 95% CI = 1.09-1.82, $P = 0.009$; additive model: adjusted OR = 1.39, 95% CI: 1.14-1.71, $P = 0.001$). No significant association was observed between the other genetic variations and gastric cancer risk (**Table 1**). To investigate the contribution of potential interactions between *IL-4/IL-4R* and *IL-6/IL-6R* to gastric cancer risk, we recalculated the combined genotypes of *IL-4/IL-4R* and *IL-6/IL-6R* genetic variations but did not find any significant associations (Tables S3 and S4).

Stratification analysis

To further assess the association between rs1800796 and the risk of gastric cancer, we performed a stratified analysis by age, gender, *H. pylori* infection status, tumor stage, and tumor site using a co-dominant model (CG/GG *vs.* CC). The increased risk of 1800796 G allele carriers (GG/CG) for gastric cancer remained significant in the following subgroups: age >64 years old (adjusted OR = 1.67, 95% CI = 1.17-2.39, $P = 0.005$), female (adjusted OR = 1.82, 95% CI = 1.09-3.05, $P = 0.023$), positive for *H. pylori* infection (adjusted OR = 1.54, 95% CI = 1.07-2.22, $P = 0.023$), non-gastric cardiac adenocarcinoma (NGCA; adjusted OR = 1.53, 95% CI = 1.15-2.04, $P = 0.003$), tumor stage T3-T4 (adjusted OR = 1.41, 95% CI = 1.06-1.88, $P = 0.020$), and median to high differentiation (adjusted OR = 1.45, 95% CI = 1.08-1.96, $P = 0.015$; **Table 2**).

Associations between genetic variations and clinical outcomes

Follow-ups were conducted with 460 gastric cancer patients to obtain survival information. To assess associations between genetic variations and prognosis, we used univariate Cox regression analysis to calculate HRs for patients with heterozygous and homozygous genotypes and to compare them with HRs for patients with the wild type genotype. We did not find any associations between the genetic variations and OS (**Table 3**), indicating that these genetic variations have no predictive value for gastric cancer.

Discussion

A total of 479 gastric cancer patients and 483 age- and gender-matched healthy controls in a Chinese population were recruited for this pop-

Table 1. Associations between *IL-4* and *IL-6* polymorphisms and gastric cancer risk

Genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)	AOR (95% CI)[a]	*p*-value
IL-4 rs2243248					
TT	421 (87.89)	420 (86.96)	Reference	Reference	
GT	57 (11.90)	62 (12.84)	0.92 (0.63, 1.35)	0.92 (0.63, 1.37)	0.693
GG	1 (0.21)	1 (0.21)	1.00 (0.06, 16.04)	0.74 (0.05, 12.11)	0.836
GT/GG	58 (12.11)	63 (13.04)	0.92 (0.63, 1.35)	0.92 (0.63, 1.35)	0.671
Additive model			0.92 (0.64, 1.34)	0.92 (0.63, 1.34)	0.660
IL-4 rs2070874					
TT	309 (64.51)	321 (66.46)	Reference	Reference	
TC	147 (30.69)	142 (29.40)	1.08 (0.81, 1.42)	1.03 (0.78, 1.37)	0.817
CC	23 (4.80)	20 (4.14)	1.19 (0.64, 2.22)	1.24 (0.66, 2.32)	0.511
TC/CC	170 (35.49)	162 (33.54)	1.09 (0.84, 1.42)	1.06 (0.81, 1.39)	0.685
Additive model			1.08 (0.87, 1.35)	1.07 (0.85, 1.34)	0.576
IL-4R rs2057768					
TT	125 (26.10)	139 (28.78)	Reference	Reference	
CT	241 (50.31)	239 (49.48)	1.12 (0.83, 1.51)	1.16 (0.86, 1.58)	0.331
CC	113 (23.59)	105 (21.74)	1.20 (0.84, 1.71)	1.17 (0.81, 1.69)	0.405
CT/CC	354 (73.90)	344 (71.22)	1.14 (0.86, 1.52)	1.17 (0.87, 1.56)	0.298
Additive model			1.10 (0.92, 1.31)	1.09 (0.91, 1.31)	0.349
IL-4R rs2107356					
CC	192 (40.08)	202 (41.82)	Reference	Reference	
TC	224 (46.76)	215 (44.51)	1.10 (0.84, 1.44)	1.15 (0.87, 1.51)	0.340
TT	63 (13.15)	66 (13.66)	1.00 (0.68, 1.50)	0.98 (0.65, 1.46)	0.908
TC/TT	287 (59.92)	281 (58.18)	1.08 (0.83, 1.39)	1.09 (0.84, 1.42)	0.500
Additive model			1.03 (0.85, 1.24)	1.02 (0.85, 1.23)	0.813
IL-4R rs1805015					
TT	402 (83.92)	404 (83.64)	Reference	Reference	
CT	76 (15.87)	76 (15.73)	1.01 (0.71, 1.42)	1.00 (0.70, 1.42)	0.994
CC	1 (0.21)	3 (0.62)	0.34 (0.04, 3.24)	0.34 (0.04, 3.29)	0.351
CT/CC	77 (16.08)	79 (16.36)	0.98 (0.70, 1.38)	0.97 (0.69, 1.38)	0.880
Additive model			0.96 (0.69, 1.33)	0.95 (0.68, 1.33)	0.754
IL-4R rs1801275					
AA	326 (68.06)	329 (68.12)	Reference	Reference	
GA	137 (28.60)	133 (27.54)	1.04 (0.78, 1.38)	1.04 (0.78, 1.38)	0.812
GG	16 (3.34)	21 (4.35)	0.77 (0.39, 1.50)	0.71 (0.36, 1.41)	0.331
GA/GG	153 (31.94)	154 (31.88)	1.00 (0.77, 1.32)	0.99 (0.75, 1.30)	0.934
Additive model			0.97 (0.77, 1.22)	0.95 (0.75, 1.20)	0.662
IL-6 rs6949149					
TT	151 (31.52)	144 (29.81)	Reference	Reference	
GT	228 (47.60)	248 (51.35)	0.88 (0.66, 1.17)	0.89 (0.66, 1.19)	0.421
GG	100 (20.88)	91 (18.84)	1.05 (0.73, 1.51)	1.06 (0.73, 1.54)	0.758
GT/GG	328 (68.48)	339 (70.19)	0.92 (0.70, 1.21)	0.93 (0.70, 1.23)	0.610
Additive model			1.01 (0.84, 1.21)	1.02 (0.85, 1.22)	0.863
IL-6 rs10499563					
TT	298 (62.21)	303 (62.73)	Reference	Reference	
TC	163 (34.03)	157 (32.51)	1.06 (0.81, 1.39)	1.08 (0.82, 1.42)	0.602
CC	18 (3.76)	23 (4.76)	0.80 (0.42, 1.51)	0.84 (0.44, 1.60)	0.601
TC/CC	181 (37.79)	180 (37.27)	1.02 (0.79, 1.33)	1.05 (0.80, 1.36)	0.747
Additive model			0.99 (0.79, 1.23)	1.01 (0.81, 1.26)	0.948

IL-6 rs1800796					
CC	226 (47.18)	268 (55.49)	Reference	Reference	
CG	203 (42.38)	187 (38.72)	1.29 (0.99, 1.68)	1.28 (0.98, 1.68)	0.071
GG	50 (10.44)	28 (5.80)	**2.12 (1.29, 3.48)**	**2.20 (1.33, 3.63)**	0.002
GC/GG	253 (52.82)	215 (44.51)	**1.40 (1.08, 1.80)**	**1.41 (1.09, 1.82)**	0.009
Additive model			**1.38 (1.13, 1.68)**	**1.39 (1.14, 1.71)**	0.001
IL-6R rs2228145					
AA	163 (34.03)	157 (32.51)	Reference	Reference	
CA	232 (48.43)	235 (48.65)	0.95 (0.72, 1.26)	0.96 (0.72, 1.29)	0.803
CC	84 (17.54)	91 (18.84)	0.89 (0.62, 1.29)	0.88 (0.61, 1.29)	0.519
CA/CC	316 (65.97)	326 (67.49)	0.93 (0.71, 1.22)	0.94 (0.72, 1.24)	0.678
Additive model			0.94 (0.79, 1.13)	0.94 (0.79, 1.13)	0.541

[a]Adjusted for age, gender, smoking, drinking, and *H. pylori* infection status. *p*-values <0.05 are shown in bold. AOR, adjusted odds ratio; OR, odds ratio.

Table 2. Stratification analysis of the associations between rs1800796 genotypes and gastric cancer risk

Variables	Rs1800796 (cases/controls)		AOR (95% CI)	*p*-value[a]
	CC	CG/GG		
Age				
≤64	115/125	96/87	1.16 (0.80, 1.70)	0.432
>64	111/143	107/100	1.67 (1.17, 2.39)	0.005
Gender				
Male	163/187	190/171	1.30 (0.96, 1.75)	0.091
Female	63/81	63/44	1.82 (1.09, 3.05)	0.023
H. pylori infection				
Positive	121/133	140/98	1.54 (1.07, 2.22)	0.019
Negative	105/135	113/117	1.28 (0.88, 1.85)	0.193
Differentiation				
Low	93/268	99/215	1.34 (0.95, 1.89)	0.091
Median to high	133/268	154/215	1.45 (1.08, 1.96)	0.015
Clinical stage				
I-II	76/268	83/215	1.39 (0.95, 2.01)	0.088
III-IV	150/268	170/215	1.41 (1.06, 1.88)	0.020
Tumor site				
Cardia	72/268	66/215	1.15 (0.78, 1.68)	0.493
Non-cardia	154/268	187/215	1.53 (1.15, 2.04)	0.003

[a]Adjusted for age, gender, smoking, drinking, and *H. pylori* infection status. AOR, adjusted odds ratio.

ulation-based case-control study. The results revealed that *IL-6* rs1800796 was associated with an increased risk of gastric cancer and that this association was maintained in the following subgroups: older age (>64 years), female, positive for *H. pylori* infection, NGCA, gastric cancer with median to high differentiation, and stage T3-T4 gastric cancer. In addition, prognostic analysis of 460 patients with survival times up to 5 years showed that none of the selected genetic variations were associated with survival of gastric cancer patients.

IL-6 is located at chromosome 7q21.3 and contains five exons and four introns. Previously, four genetic variations (-174G/C, rs1800795; -572 or -634G/C, rs1800796; -597A/G, rs1800797; and -6331T/C, rs10499563) in the promoter region were associated with a risk of gastric cancer [16, 17, 26]. However, two studies of these variations in the Chinese population have shown conflicting conclusions [14, 15]; this inconsistency is possibly due to the different populations (northern or southern Chinese populations) evaluated or due to the different sample sizes of their studies (215 *vs.* 375 patients). To attempt to resolve this inconsistency, we evaluated the associations between genetic variations and gastric cancer risk in a mid-east Chinese Han population. We showed that *IL-6* rs1800796 was associated with gastric cancer risk, and this is consistent with the results of a meta-analysis that also show that the rs1800796GG genotype is associated with an increased risk of cancer [13, 19, 20, 27]; specifically, a significant association was ob-

Table 3. Analysis of associations between genetic variations and clinical outcomes

Genotype	Cases, n	Death, n (%)	Log-rank *p*-value	HR
IL-4 rs2248				
TT	406	255 (0.63)		Reference
GT/GG	54	31 (0.57)	0.734	1.07 (0.74, 1.55)
IL-4 rs2070874				
TT	301	192 (0.64)		Reference
TC/CC	159	94 (0.59)	0.417	1.11 (0.87, 1.42)
IL-4R rs2057768				
TT	119	72 (0.61)		Reference
CT/CC	341	214 (0.63)	0.451	1.05 (0.92, 1.20)
IL-4R rs2107356				
CC	182	108 (0.59)		Reference
TC/TT	278	178 (0.64)	0.317	1.13 (0.89, 1.44)
IL-4R rs1805015				
TT	388	241 (0.62)		Reference
CT/CC	72	45 (0.63)	0.745	1.03 (0.88, 1.20)
IL-4R rs1801275				
AA	316	197 (0.62)		Reference
GA/GG	144	89 (0.62)	0.843	1.03 (0.80, 1.32)
IL-6 rs6949149				
TT	144	88 (0.61)		Reference
GT/GG	316	198 (0.63)	0.510	1.04 (0.92, 1.18)
IL-6 rs10499563				
TT	286	183 (0.64)		Reference
TC/CC	174	103 (0.59)	0.417	1.11 (0.87, 1.41)
IL-6 rs1800796				
CC	220	137 (0.62)		Reference
GC/GG	240	149 (0.62)	0.952	1.00 (0.89, 1.13)
IL-6R rs2228145				
AA	156	96 (0.62)		Reference
CA/CC	304	190 (0.63)	0.873	1.02 (0.80, 1.30)

HR was estimated using the univariate Cox regression model.

served in the pooled results of the Asian population [13, 28] and with the results of a study on genetic variations and cancer risk in a Korean population [29]. These studies also did not show a significant association between *IL-6* s1800796GG and the risk of gastric cancer [18, 23, 27, 30, 31], possibly because of the occurrence of different frequencies of the rs1800796G allele in different races. The frequency of the rs1800796G allele is known to be 23.9% in an Asian population and 93.8% in a European population [27]. In this study, we found that the frequency of the rs1800796G allele was 25.15% in a Chinese population. Moreover, the latest meta-analysis concluded that rs1800796 is associated with cancer risk in Asians, but not in Caucasians [32]. However, for gastric cancer, one study with limited samples reported a negative result in Caucasians [33]. Thus, such an association is unclear in Caucasians and should be investigated further. It has also been shown that the *IL-6* rs1800796 genetic variation is associated with the level of IL-6 in serum [34]. Because the rs1800796 genetic variation is in the promoter region of *IL-6*, it could affect the rate of transcription; the C allele genetic variant is associated with increased transcription of IL-6 when compared with the G allele [35, 36]. In addition, individuals carrying the GG genotype are found to have higher IL-6 levels [37, 38]. Further, circulating blood levels of IL-6 have been shown to function as potential diagnostic biomarkers for gastric cancer [39, 40].

In the stratification analysis, we observed that the carriers of the rs1800796G allele (GG/CG) in the older age (>64 years), female, and 'positive for *H. pylori* infection' subgroups had an increased risk of gastric cancer. It is well known that older individuals have longer exposures to risk factors, which enhances the risk of gastric cancer. The association between the genetic variations and gastric cancer risk observed in the female subgroup may be due to differences in the lifestyles of females and males; for example, females may smoke and drink less than males. For the individuals that were positive for *H. pylori* infection, rs1800796 was associated with an increased risk of gastric cancer, and this is consistent with the fact that *H. pylori* infection is a risk factor for gastric cancer. The subgroup analysis also revealed that the rs1800796 allele was associated with NGCA, stage T3-T4 cancer, and cancer with median to high differentiation, thus indicating that the association between the rs1800796 allele and the risk of gastric cancer could be affected by the pathological characteristics of gastric cancer.

We observed no significant association between the genetic variations and the prognosis of gastric cancer. Although serum IL-6 levels have been associated with survival of gastric cancer patients [41] and *IL-6* genetic variations (rs1800796, rs8192284) have been shown to affect the prognosis and OS of patients with gastric cancer [21], we did not observe a significant association between rs1800796 and OS in our study. This may be due to differences in sample sizes (the previously published study involved 161 gastric cancer patients) and the different MAFs of rs1800796 in Asian and European populations.

Although similar findings have been previously reported, a novelty of this study is that we analyzed genetic variations involved in the IL-4/IL-4R and IL-6/IL-6R pathways in gastric cancer. In addition, we conducted a subgroup analysis based on clinical characteristics (age, gender, and *H. pylori* infection status) and pathological characteristics (differentiation, clinical stage, and tumor site). More importantly, we evaluated the prognostic value of these genetic variations using data from 460 Chinese patients who were followed up for up to five years. Nevertheless, some limitations of this study should be noted. First, the relatively small sample size may have limited the statistical power, especially in the multiple stratified analysis. Further, studies with a larger sample size are required to confirm our findings. Second, several environmental factors, such as diet and history of gastric disease, were not included in this study and may influence gastric cancer risk. Such an influence could be minimized by more restrictive case selection criteria. Third, the associations between some of the genetic variations and gastric cancer risk have been identified previously, which may weaken the novelty of this study. However, even though such associations in our Chinese population are unclear, the results of this study will inform future research in this area. Finally, the genetic variations included in this study were selected based on their potential functional roles in cancer occurrence, but their functions remain unclear. Therefore, the functions of the genetic variations require further elucidation.

In conclusion, this case-control study demonstrated that the *IL-6* rs1800796 allele *IL-6* associates with gastric cancer risk, and the association is stronger in individuals that are >64 years old, female, or positive for *H. pylori* infection. None of the genetic variations that we analyzed are associated with the prognosis of gastric cancer.

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Disclosure of conflict of interest

None.

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Table S1. Information on the included genetic variations

Gene	SNP ID	Chromosome position	Allele	Position	HWE
IL4	rs2243248	Chr 5: 132672952	T/G	Promoter, -1098T>G	0.410
IL4	rs2070874	Chr 5: 132674018	T/C	5'UTR, Ex1-168T>C	0.396
IL4R	rs2057768	Chr 16: 27310774	C/T	Promoter, -3223C>T	0.905
IL4R	rs2107356	Chr 16: 27312083	C/T	Promoter, -1914C>T	0.467
IL4R	rs1805015	Chr 16: 27362859	T/C	Ex12, Ser503Pro	0.778
IL4R	rs1801275	Chr 16: 27363079	A/G	Ex12, Gln576Arg	0.114
IL6	rs6949149	Chr 7: 22709538	T/G	Promoter, -17724T>G	0.386
IL6	rs10499563	Chr 7: 22720869	T/C	Promoter, -6331T>C	0.647
IL6	rs1800796	Chr 7: 22726627	C/G	Promoter, -572C>G	0.536
IL6R	rs2228145	Chr 1: 154454494	A/C	Ex9, Asp358Ala	0.854

MAF: minor allele frequency, 5'FR: 5' flanking region, 3'UTR: 3' untranslated region, Ex: exon, HWE, Hardy-Weinberg equilibrium.

Table S2. Clinical and demographic characteristics of the enrolled participants

Variables	Cases, n (%)	Healthy controls, n (%)	*p*-value
All subjects	479	483	
Age (Mean ± SD)	64.48 ± 11.91	64.73 ± 11.84	
Gender			
Male	353 (73.70)	358 (74.12)	0.881
Female	126 (26.30)	125 (25.88)	
H. pylori infection			
Positive	261 (54.49)	231 (47.83)	0.039
Negative	218 (45.51)	252 (52.17)	
Cigarette smoking			
Never	368 (76.83)	419 (86.75)	<0.001
Ever	111 (23.17)	64 (13.25)	
Alcohol consumption			
No	426 (88.94)	461 (95.45)	<0.001
Yes	53 (11.06)	22 (4.55)	
TNM stage			
I-II	159 (33.19)		
III-IV	320 (66.81)		
Tumor site			
Cardia	138 (28.81)		
Non-cardia	341 (71.19)		

Table S3. Locus-locus interaction between *Il-4/IL-4R* and gastric cancer risk

IL-4	*IL-4R*	Cases	Controls	OR (95% CI)	OR (95% CI)[a]	*p*-value
rs2243248	rs20577768					
TT	TT	113	121	Reference	Reference	
TT	CT/CC	308	299	1.10 (0.82, 1.49)	1.14 (0.84, 1.55)	0.417
GT/GG	TT	12	18	0.71 (0.33, 1.55)	0.70 (0.32, 1.55)	0.383
GT/GG	CT/CC	46	45	1.10 (0.67, 1.78)	1.10 (0.68, 1.80)	0.696
rs2243248	rs2107356					
TT	CC	169	176	Reference	Reference	
TT	TC/TT	252	244	1.08 (0.82, 1.42)	1.11 (0.84, 1.47)	0.465
GT/GG	CC	23	26	0.92 (0.51, 1.68)	0.92 (0.50, 1.68)	0.777
GT/GG	TC/TT	35	37	0.99 (0.59, 1.64)	0.99 (0.59, 1.66)	0.969
rs2243248	rs1805015					
TT	TT	359	350	Reference	Reference	
TT	CT/CC	62	70	0.86 (0.60, 1.25)	0.86 (0.59, 1.25)	0.422
GT/GG	TT	43	54	0.78 (0.51, 1.19)	0.77 (0.50, 1.18)	0.232
GT/GG	CT/CC	15	9	1.63 (0.70, 3.76)	1.68 (0.72, 3.92)	0.233
rs2243248	rs1801275					
TT	AA	292	283	Reference	Reference	
TT	GA/GG	129	137	0.91 (0.68, 1.22)	0.89 (0.66, 1.20)	0.449
GT/GG	AA	34	46	0.72 (0.45, 1.15)	0.69 (0.43, 1.12)	0.134
GT/GG	GA/GG	24	17	1.37 (0.72, 2.60)	1.42 (0.74, 2.71)	0.290
rs2070874	rs20577768					
TT	TT	84	91	Reference	Reference	
TT	CT/CC	225	230	1.06 (0.75, 1.50)	1.10 (0.77, 1.56)	0.614
TC/CC	TT	41	48	0.93 (0.56, 1.54)	0.86 (0.51, 1.45)	0.572
TC/CC	CT/CC	129	114	1.23 (0.83, 1.81)	1.18 (0.79, 1.75)	0.417
rs2070874	rs2107356					
TT	CC	125	128	Reference	Reference	
TT	TC/TT	184	193	0.98 (0.71, 1.34)	1.02 (0.74, 1.41)	0.906
TC/CC	CC	67	74	0.93 (0.61, 1.40)	0.92 (0.61, 1.40)	0.694
TC/CC	TC/TT	103	88	1.20 (0.82, 1.75)	1.17 (0.80, 1.71)	0.425
rs2070874	rs1805015					
TT	TT	264	263	Reference	Reference	
TT	CT/CC	45	58	0.77 (0.51, 1.18)	0.76 (0.49, 1.17)	0.209
TC/CC	TT	138	141	0.98 (0.73, 1.30)	0.94 (0.70, 1.27)	0.690
TC/CC	CT/CC	32	21	1.52 (0.85, 2.70)	1.49 (0.83, 2.68)	0.182
rs2070874	rs1801275					
TT	AA	215	209	Reference	Reference	
TT	GA/GG	94	112	0.82 (0.58, 1.14)	0.79 (0.56, 1.10)	0.165
TC/CC	AA	111	120	0.90 (0.65, 1.24)	0.87 (0.62, 1.20)	0.385
TC/CC	GA/GG	59	42	1.37 (0.88, 2.12)	1.34 (0.85, 2.09)	0.205

[a]Adjusted for age, gender, smoking, drinking, and *H. pylori* infection status.

Table S4. Locus-locus interaction between *Il-6*/*IL-6R* and gastric cancer risk

IL-6	*IL-6R*	Cases	Controls	OR (95% CI)	OR (95% CI)[a]	*p*-value
rs6949149	rs2228145					
TT	AA	49	53	Reference	Reference	
TT	CA/CC	102	91	1.21 (0.75, 1.96)	1.28 (0.78, 2.09)	0.333
GT/GG	AA	114	104	1.19 (0.74, 1.90)	1.26 (0.78, 2.03)	0.353
GT/GG	CA/CC	214	235	0.99 (0.64, 1.52)	1.02 (0.66, 1.59)	0.923
rs10499563	rs2228145					
TT	AA	98	97	Reference	Reference	
TT	CA/CC	200	206	0.96 (0.68, 1.35)	0.98 (0.69, 1.39)	0.911
TC/CC	AA	65	60	1.07 (0.68, 1.68)	1.16 (0.73, 1.84)	0.526
TC/CC	CA/CC	116	120	0.96 (0.66, 1.40)	0.99 (0.67, 1.46)	0.953
rs1800796	rs2228145					
CC	AA	88	96	Reference	Reference	
CC	CA/CC	138	172	0.88 (0.61, 1.26)	0.92 (0.63, 1.33)	0.650
CG/GG	AA	75	61	1.34 (0.86, 2.09)	1.43 (0.90, 2.26)	0.128
CG/GG	CA/CC	178	154	1.26 (0.88, 1.81)	1.29 (0,89, 1.87)	0.182

[a]Adjusted for age, gender, smoking, drinking, and *H. pylori* infection status.