Association of *IL10* gene promoter polymorphisms with risks of gastric cancer and atrophic gastritis

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Abstract

Objective: To investigate the association between polymorphisms of the interleukin 10 (*IL10*) gene and risk of gastric cancer (GC) and atrophic gastritis (AG).

Methods: This study enrolled patients with GC, patients with AG and healthy control subjects. Demographic data were collected and the *IL10* gene −1082A/G, −819C/T and −592A/C polymorphisms were genotyped. An enzyme-linked immunosorbent assay was performed to detect *Helicobacter pylori* infection.

Results: The study enrolled 556 participants including 208 in the GC group, 116 in the AG group and 232 controls (CON group). In a recessive model of the *IL10*–819C/T polymorphism, a significantly decreased risk of GC was found compared with AG and non-cancer subjects, respectively (AG→GC: odds ratio OR 0.41; non-cancer→GC: OR 0.57). The CC genotype demonstrated a significantly increased risk of AG compared with CON. Similar significant results were detected in males and *H. pylori*-negative subgroups. The ACC haplotype was associated with a decreased risk of GC compared with AG. The ATC haplotype was associated with a decreased risk of AG compared with the CON group, but it was associated with an increased risk of GC compared with AG.

Conclusion: The *IL10* gene promoter −819C/T (rs1800871) polymorphism was associated with the risk of GC and AG in a Chinese population.

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Introduction

Gastric cancer (GC), one of the most common cancers of the digestive tract worldwide, is the second leading cause of cancer-related deaths.[1] As a complex and multistep process, GC is initiated by both genetic and environmental factors with their complicated interactions.[2] Although environmental factors including *Helicobacter pylori* infection have been identified as risk factors for GC, genetic influences and their interactions with environmental factors also play an important role in gastric carcinogenesis.[3] Gene polymorphisms are the basis of the diversity of individual genetic susceptibility, of which single nucleotide polymorphisms (SNPs) are the most common form of genetic variation. Therefore, investigation and identification of the SNPs associated with susceptibility to GC and its precancerous diseases are critical to unrabel the disease aetiology and identify promising biomarkers and therapeutic targets for GC.[4]

Inflammatory and immune reactions have been suggested to be implicated in the occurrence and progression of various types of cancers including GC.[5] Cytokines are secretory proteins that are involved in the regulation of various biological activities including haematopoiesis, inflammation and immunity.[6] Interleukin (IL)-10 is a cytokine with multiple biological effects covering anti-inflammation and antiallergy.[7] Also, IL-10 is an immunosuppressive cytokine involved in the initiation and progression of cancer.[8] It has been reported that the over-expression of IL-10 suppressed the phagocytosis of macrophage effect of cells, thus contributing to the spread of cancer cells.[9] In a study in which the mouse *IL10* gene was transfected into human melanoma cells, IL-10 inhibited cancer cell growth as well as decreasing metastasis and antiangiogenesis.[10]

Gastric cancer progresses stepwise from normal stomach tissue through inflammatory and precancerous conditions ultimately to cancer, as described by Correa's cascade.[11] *Helicobacter pylori* infection is the most critical risk factor for the development of GC.[12] However, the outcome of *H. pylori* carriers differ among different individuals, indicating that host genetic factors are also implicated in the determination of clinical outcome.[13] The interaction between host genetic factors and *H. pylori* infection is the focus of considerable research. Polymorphisms of several genes including *COX-2*, *IL-8*, *IL-1B* and *PGC* have been reported to demonstrate interactions with *H. pylori*.[14–18] As an inflammation-related cytokine, *IL10* gene polymorphisms might be involved in gastric carcinogenesis and interact with *H. pylori*.

In this study, three polymorphisms (–1082A/G, –819C/T, –592A/C) of the *IL10* gene promoter region were investigated for their role in the susceptibility of GC. Their interactions with *H. pylori* were also studied to elucidate the relationship between *IL10* gene polymorphisms and this environmental factor. In addition, haplotype analysis was conducted to reveal the synergistic effect of these polymorphisms.

Patients and methods

Study population

This study enrolled patients with gastric cancer (GC group), patients with atrophic gastritis (AG group) and healthy control subjects (CON group) from The First Hospital of China Medical University, Shenyang, China and the Zhuanghe Gastric Diseases Screening Programme[19] between March 2002 and December 2011. All the patients had undergone a surgical operation or gastroscopic examinations and were diagnosed according to the updated Sydney gastritis classification[20,21] and the World Health Organization classification of tumours of the digestive system.[22] There were two exclusion criteria for GC patients: (i) having a history of another malignant neoplasm; (ii) accepting preoperative chemotherapy or radiotherapy. The control subjects were recruited from the Zhuanghe Gastric Diseases Screening Programme and were diagnosed with a normal stomach or only superficial gastritis on the basis of gastroscopic and histopathological examinations. Other information such as sex and age of the enrolled participants was extracted from registered documents.

The study was approved by the Human Ethics Committee of The First Hospital of China Medical University, Shenyang, China. Each participant involved in the study provided written informed consent.

Genotyping of IL10 gene −1082A/G, −819C/T and −592A/C polymorphisms

A 5-ml sample of whole blood was collected from each study participant to isolate DNA. The blood samples were allowed to clot for 30 min to 1 h at room temperature and then centrifuged at 3500 g using a benchtop multi-function cryopreservation centrifuge GS-15R (Beckman Coulter Life Sciences, Indianapolis, IN, USA) at 20–25°C for 5 min. The clot was transferred to a 2 ml centrifuge tube, stored immediately at –20°C, and then moved into a freezer at −80°C until DNA extraction. IL10 gene −1082A/G, −819C/T and −592A/C polymorphisms were genotyped using polymerase chain reaction (PCR)–restriction fragment length polymorphism technology. The primer sequences were taken from previously published work.[23–26] The primers were as follows: IL10 gene −1082A/G (rs1800896) polymorphism: forward: 5′-TCT TAC CTA TCC CTA CTT CC- 3′, reverse: 5′-CTC GCT GCA ACC CAA CTG GC-3′; IL10 gene −819C/T (rs1800871) polymorphism: forward: 5′-CAC TAC TAA GGC TTC CTT GGG A-3′, reverse: 5′-GTG AGC AAA CTG AGG CAC GAC AT-3′; IL10 gene −592A/C (rs1800872) polymorphism: forward: 5′-GGT GAG CAC TAC CTG ACT AGC-3′, reverse: 5′-CCT AGG TCA CAG TGA CGT GGG-3′. Genomic DNA was purified using a method described previously with some modifications.[27,28] The primers were purchased from TaKaRa Biotechnology (Dalian, China). The total PCR reaction volume was 25 μl containing 2.5 μl of 10 × PCR buffer, 2.0 μl of 2.5 mM dNTP mixture, 1.0 μl of each upstream and downstream primer at 10 pmol/μl, 2.5 U或0.5 μL, 1.0 μl of the template DNA (TaKaRa Biotechnology), and an appropriate amount of ddH$_2$O. The PCR reaction was performed by preliminary denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 45 s; and 72°C for 40 s and then 72°C for 60 s using a Biometra TGradient 96 thermal cycler (Analytik Jena, Jena, Germany). Restriction enzyme MnlI (Fermentas, Waltham, MA, USA) was used to digest 10 μl of PCR product overnight for genotyping the IL10 gene −1082A/G polymorphism, generating a 139 base pair (bp) fragment for the AA genotype, and 106 bp, 33 bp fragments for the GG

genotype. Restriction enzyme *Hin1*II (Fermentas) was used for genotyping the *IL10* gene –819C/T polymorphism, generating 232 bp and 78 bp fragments for the TT genotype, and 211 bp, 78 bp and 21 bp fragments for the CC genotype. Restriction enzyme Rsa I (Fermentas) was used for genotyping the *IL10* gene –592A/C polymorphism, generating 236 bp and 176 bp fragments for the AA genotype, and a 412 bp fragment for the CC genotype. The *IL10* gene –1082 and *IL10* gene –592 PCR products (20 µl) were separated using 3% agarose gel by electrophoresis at 150 V for 30 min, then stained using ethidium bromide for 5 min and observed using an ABI 377 DNA Sequencer (SeqGen, Torrance, CA, USA). The *IL10* gene –819 PCR products (5 µl) were separated in 10% polypropylene gel and underwent electrophoresis at 120 V for 20 min followed by 80 V for 40 min, then stained using ethidium bromide for 5 min and observed using an Gel automatic imaging system GDS800 (BIO-RAD, Hercules, CA, USA). Approximately 10% of the samples were confirmed by DNA sequencing using an ABI 377 DNA Sequencer (SeqGen, Torrance, CA, USA) in this study.

Helicobacter pylori infection status

Approximately 5 ml of venous blood was collected from each participant after an overnight fast and the serum was obtained after 10 min centrifugation at 1000 g at room temperature using a benchtop multifunction cryopreservation centrifuge GS-15R (Beckman Coulter Life Sciences). The serum was used to undertake a serology test to determine the *H. pylori* infection status using an enzyme-linked immunosorbent assay (ELISA) that measured *H. pylori* immunoglobin (Ig) G levels (*H. pylori*-Ig G ELISA kit; BIOHIT Healthcare, Helsinki, Finland) according to the manufacturer's instructions as described previously.[29] A numerical reading more than

34 enzyme immune units was regarded as a *H. pylori* infection positive result.

Statistical analyses

All statistical analyses were performed using the SPSS® statistical package, version 16.0 (SPSS Inc., Chicago, IL, USA) for Windows®. The differences in age between the different groups were assessed using analysis of variance. The χ^2-test was used to investigate differences between categorical variables including sex and *H. pylori* infection status. The association between each SNP and the risk of AG and GC was estimated by calculating odds ratios (ORs) and their 95% confidence intervals (CIs) using multivariate logistic regression adjusting for sex, age and *H. pylori* infection status. Stratified analysis by sex and *H. pylori* infection status was also performed. Interactions between SNPs and the environment were investigated by including both the main effect variables and their product terms in the logistic regression models. Haplotype association analyses were performed using SHEsis online software.[30,31] A *P*-value < 0.05 was considered statistically significant.

Results

This study enrolled a total of 556 individuals including 208 patients with GC (GC group), 116 patients with AG (AG group) and 232 healthy control subjects (CON group). The GC group had a higher proportion of males (139 of 208; 66.8%) compared with the AG (68 of 116; 58.6%) and CON groups (136 of 232; 58.6%) but the differences were not significant (Table 1). Based on participants that had serum samples for testing, the *H. pylori* infection rates in the GC group (50 of 145; 34.5%) and the AG group (44 of 113; 38.9%) were significantly higher than that of the CON group (57 of 208; 27.4%) (*P* < 0.001).

Table 1. Baseline demographic characteristics of patients with gastric cancer (GC group), patients with atrophic gastritis (AG) and healthy control subjects (CON group) who participated in a study of interleukin 10 gene promoter polymorphisms.

	GC group	AG group	CON group	Statistical significance[a]
Characteristic	208	116	232	
Age, years	58.74 ± 11.65	56.51 ± 10.74	52.27 ± 13.84	$P < 0.001$
Age range, years	30–84	32–79	19–84	
Sex				NS
Male	139 (66.8%)	68 (58.6%)	136 (58.6%)	
Female	69 (33.2%)	48 (41.4%)	96 (41.4%)	
Helicobacter pylori infection[b]				$P < 0.001$
Positive	50 (34.5%)	44 (38.9%)	57 (27.4%)	
Negative	95 (65.5%)	69 (61.1%)	151 (72.6%)	

Data presented as mean ± SD or *n* of patients (%).
[a]Age was compared using analysis of variance; χ^2-test was used to compare categorical variables; NS, no significant between-group difference ($P \geq 0.05$).
[b]Participants with serum samples available.

The results of the association between polymorphisms (–1082A/G, –819C/T and –592A/C) of the *IL10* gene promoter region and disease risk are summarized in Table 2. No significant relationship was found between the AG genotype of the *IL10* gene –1082A/G polymorphism and risks of AG or GC compared with the CON group (AG: OR 1.89, 95% CI 0.90, 3.98; GC: OR 1.69, 95% CI 0.86, 3.33). The GG genotype frequency was rare in all three groups. The dominant and recessive genetic model did not demonstrate a significant result.

For the *IL10* gene –819 C/T polymorphism, the CC genotype was associated with a decreased risk in AG→GC compared with the TT genotype (OR 0.42, 95% CI 0.21, 0.83, $P = 0.012$) (Table 2). In the recessive genetic model, a significant decreased risk of GC was found compared with AG and non-cancer subjects, respectively (AG→GC: OR 0.41, 95% CI 0.22, 0.75, $P = 0.004$; non-cancer→GC: OR 0.57, 95% CI 0.34, 0.96, $P = 0.034$). In addition, CC genotype carriers demonstrated a significantly increased risk of AG compared with CON (OR 1.79, 95% CI 1.02, 3.13, $P = 0.043$).

For the *IL10* gene –592A/C polymorphism, no significant association was suggested in the heterozygous, homozygous, dominant or recessive genetic models.

A subgroup investigation was undertaken to further elucidate the effect of sex and *H. pylori* on the results of the association analysis (Table 3). In the recessive genetic model of the *IL10* gene –819C/T polymorphism, the CC genotype was significantly associated with a decreased risk of GC in males compared with AG and non-cancer, respectively (AG→GC: OR 0.35, 95% CI 0.16, 0.78, $P = 0.010$; non-cancer→GC: OR 0.45, 95% CI 0.23, 0.89, $P = 0.022$). In the subgroup analysis of *H. pylori*-negative participants, the CC genotype also demonstrated a significantly decreased GC risk (AG→GC: OR 0.33, 95% CI 0.14, 0.79, $P = 0.013$; non-cancer→GC: OR 0.41, 95% CI 0.19, 0.90, $P = 0.027$).

An interaction analysis was performed in order to reveal whether *IL10* gene promoter polymorphisms have an interactive effect with *H. pylori* (Table 4).

Table 2. Association between interleukin 10 gene single nucleotide polymorphisms (SNP) and disease risk in patients with gastric cancer (GC group), patients with atrophic gastritis (AG) and healthy control subjects (CON group).

SNP	Gastric mucosa status			Compared genotype	CON→AG		AG→GC		CON→GC		Non-cancer→GC	
	GC (%)	AG (%)	CON (%)		OR (95% CI)	Statistical significance	OR (95% CI)	Statistical significance	OR (95% CI)	Statistical significance	OR (95% CI)	Statistical significance
−1082	184 (88.5)	101 (87.1)	212 (91.8)	AA	ref		ref		ref		ref	
	22 (10.6)	15 (12.9)	18 (7.8)	AG	1.89 (0.90, 3.98)	NS	0.83 (0.41, 1.69)	NS	1.69 (0.86, 3.33)	NS	1.30 (0.72, 2.32)	NS
	2 (1)	0 (0)	1 (0.4)	GG	–	–	–	–	1.30 (0.11, 15.39)	NS	2.18 (0.19, 25.40)	NS
				Dominant	1.78 (0.85, 3.73)	NS	0.90 (0.45, 1.81)	NS	1.68 (0.87, 3.24)	NS	1.34 (0.76, 2.37)	NS
				Recessive	–	–	–	–	1.38 (0.12, 16.28)	NS	2.37 (0.20, 27.46)	NS
−819	85 (40.9)	41 (35.3)	92 (39.7)	TT	ref		ref		ref		ref	
	100 (48.1)	47 (40.5)	104 (44.8)	CT	1.05 (0.63, 1.76)	NS	1.06 (0.63, 1.77)	NS	1.16 (0.76, 1.76)	NS	1.10 (0.76, 1.62)	NS
	23 (11.1)	28 (24.1)	36 (15.5)	CC	1.79 (0.96, 3.34)	NS	0.42 (0.21, 0.83)	$P=0.012$	0.77 (0.41, 1.43)	NS	0.60 (0.34, 1.05)	NS
				Dominant	1.25 (0.78, 2.01)	NS	0.82 (0.51-1.32)	NS	1.06 (0.71, 1.57)	NS	0.95 (0.67, 1.36)	NS
				Recessive	1.79 (1.02, 3.13)	$P=0.043$	0.41 (0.22, 0.75)	$P=0.004$	0.71 (0.40, 1.27)	NS	0.57 (0.34, 0.96)	$P=0.034$
−592	76 (36.5)	39 (33.6)	84 (36.2)	AA	ref		ref		ref		ref	
	92 (44.2)	46 (39.7)	96 (41.4)	AC	1.06 (0.63, 1.79)	NS	1.04 (0.62, 1.77)	NS	1.16 (0.75, 1.79)	NS	1.10 (0.74, 1.64)	NS
	40 (19.2)	31 (26.7)	52 (22.4)	CC	1.36 (0.75, 2.48)	NS	0.68 (0.37, 1.26)	NS	0.94 (0.55, 1.62)	NS	0.84 (0.52, 1.37)	NS
				Dominant	1.18 (0.73, 1.89)	NS	0.90 (0.56, 1.46)	NS	1.09 (0.73, 1.62)	NS	1.01 (0.70, 1.46)	NS
				Recessive	1.35 (0.80, 2.27)	NS	0.66 (0.39, 1.14)	NS	0.87 (0.54, 1.40)	0.558	0.79 (0.52, 1.22)	NS

NS, no significant association ($P \geq 0.05$).

Table 3. Subgroup analysis of the association between interleukin 10 gene −819 polymorphism and disease risk in patients with gastric cancer (GC group), patients with atrophic gastritis (AG) and healthy control subjects (CON group).

Group	GC	AG	CON	Compared genotype	CON→AG OR (95% CI)	Statistical significance	AG→GC OR (95% CI)	Statistical significance	CON→GC OR (95% CI)	Statistical significance	Non-cancer→GC OR (95% CI)	Statistical significance
IL10 −819 CC versus CT+TT												
All	185	88	196	CT+TT	ref		ref		ref		ref	
	23	28	36	CC	1.79 (1.02, 3.13)	P=0.043	0.41 (0.22, 0.75)	P=0.004	0.71 (0.40, 1.27)	NS	0.57 (0.34, 0.96)	P=0.034
					P_interaction = 0.395*							
Sex												
Male	126	52	113	CT+TT	ref.		ref.		ref.		ref.	
	13	16	23	CC	1.55 (0.75, 3.21)	NS	0.35 (0.16, 0.78)	P=0.010	0.52 (0.25, 1.09)	NS	0.45 (0.23, 0.89)	P=0.022
Female	59	36	83	CT+TT	ref.		ref.		ref.		ref.	
	10	12	13	CC	2.20 (0.90, 5.38)	NS	0.50 (0.19, 1.28)	NS	1.20 (0.48, 3.02)	NS	0.81 (0.36, 1.84)	NS
Hp infection												
Positive	42	33	50	CT+TT	ref.		ref.		ref.		ref.	
	8	11	7	CC	2.69 (0.92, 7.84)	NS	0.57 (0.20, 1.59)	NS	1.70 (0.55, 5.31)	NS	0.97 (0.38, 2.46)	NS
Negative	9	17	26	CT+TT	ref.		ref.		ref.		ref.	
	86	52	125	CC	1.55 (0.77, 3.14)	NS	0.33 (0.14, 0.79)	P=0.013	0.47 (0.20, 1.10)	NS	0.41 (0.19, 0.90)	P=0.027

Hp, *Helicobacter pylori*; NS, no significant association (P ≥ 0.05).

Table 4. Interaction between interleukin 10 gene −819 polymorphism and *Helicobacter pylori* (Hp) infection in patients with gastric cancer (GC group), patients with atrophic gastritis (AG) and healthy control subjects (CON group).

Genotype	CON→AG		AG→GC		CON→GC	
	Hp positive	Hp negative	Hp positive	Hp negative	Hp positive	Hp negative
IL10 −819						
CT+TT	1(ref)	1.56 (0.77, 3.16)	1(ref)	0.33 (0.14, 0.81)	1(ref)	0.48 (0.21, 1.09)
CC	1.59 (0.91, 2.79)	4.31 (1.55, 12.00)	0.75 (0.42, 1.34)	0.45 (0.17, 1.22)	1.16 (0.69, 1.95)	2.13 (0.71, 6.33)
	P_interaction = 0.395*		P_interaction = 0.387*		P_interaction = 0.061*	

Data presented as odds ratio (95% confidence interval).
*No significant association (P ≥ 0.05).

No significant interaction was observed between the *IL10* gene –819 C/T polymorphism and *H. pylori* in CON→AG ($P_{interaction} = 0.395$), AG→GC ($P_{interaction} = 0.387$) or CON→GC ($P_{interaction} = 0.061$) under the recessive model.

Haplotype analysis was then performed to investigate the combined effect of these three promoter polymorphisms using SHEsis online software. Haplotypes with a frequency less than 1% were ignored in this software and the results are summarized in Table 5. It is suggested that ACC haplotype was associated with a significantly decreased risk of GC compared with AG (OR 0.70, 95% CI 0.50, 0.98, $P = 0.038$). The ATC haplotype was associated with a decreased risk of AG compared with CON (OR 0.35, 95% CI 0.13, 0.93, $P = 0.028$), but associated with an increased risk of GC compared with AG (OR 3.21, 95% CI 1.19, 8.65, $P = 0.015$). No significant relationship was detected between the other haplotypes and disease risk.

Discussion

As an aggressive malignant tumour, GC demonstrates a high incidence and poor prognosis. Identification of reliable biomarkers associated with altered GC risk has long been a research goal to improve early detection of the disease.[32] In this present study, *IL10* gene –1082A/G (rs1800896), –819C/T (rs1800871) and –592A/C (rs1800872) polymorphisms were investigated in relation to the risks of GC and AG. The interaction of *IL10* gene polymorphisms and *H. pylori* in gastric carcinogenesis was assessed and a haplotype analysis was also undertaken. The results of the recessive model (CC versus CT+TT) of the –819C/T polymorphism indicated significantly decreased risk of GC compared with AG and non-cancer subjects, respectively. In addition, the ACC haplotype was associated with a

Table 5. Haplotype analysis of the association between interleukin 10 gene –819 polymorphism and disease risk in patients with gastric cancer (GC group), patients with atrophic gastritis (AG) and healthy control subjects (CON group).

Haplotype	Gastric mucosa status			CON→AG		AG→GC		CON→GC		Non-cancer→GC	
	GC	AG	CON	OR (95% CI)	Statistical significance	OR (95% CI)	Statistical significance	OR (95% CI)	Statistical significance	OR (95% CI)	Statistical significance
ATA	240.07	124.00	257.26	0.89 (0.65, 1.22)	NS	1.21 (0.88, 1.68)	NS	1.08 (0.83, 1.41)	NS	1.12 (0.88, 1.44)	NS
ACC	123.94	88.24	155.42	1.19 (0.86, 1.65)	NS	0.70 (0.50, 0.98)	P = 0.038	0.83 (0.63, 1.11)	NS	0.79 (0.60, 1.02)	NS
ATC	25.99	4.76	26.16	0.35 (0.13, 0.93)	P = 0.028	3.21 (1.19, 8.65)	P = 0.015	1.11 (0.63, 1.94)	NS	1.43 (0.84, 2.45)	NS
GCC	22.06	14.76	17.42	1.87 (0.68, 5.14)	NS	0.83 (0.42, 1.64)	NS	1.43 (0.75, 2.71)	NS	1.15 (0.66, 2.01)	NS

NS, no significant association ($P \geq 0.05$).

significantly decreased risk of GC compared with AG; and the ATC haplotype was associated with a decreased risk of AG compared with CON, but an increased risk of GC compared with AG. No significant interaction between *IL10* promoter polymorphisms and *H. pylori* was found.

Cytokines have been reported to participate in the regulation of the inflammatory response of the gastric mucosa.[33] The *IL10* gene is mapped to chromosome 1q31-q32, encoding a cytokine with pleiotropic effects in immunoregulation and inflammation.[34] For example, IL-10 inhibits the production of proinflammatory cytokines and downregulates the inflammatory response.[35] Genetic variants may play an important role in the pathogenesis of GC and AG.[36] Polymorphisms of the promoter region in the *IL10* gene might influence the transcription and function of IL-10, thus altering individual susceptibility to GC. Previously, the *IL10* gene (–819) polymorphism was found to be associated with an enhanced risk of peptic ulcer disease in *H. pylori*-positive patients.[37] In addition, the *IL10* –819C and –592C alleles were associated with an increased risk of intestinal-type noncardia GC in *H. pylori*-positive subjects and current smokers.[38] Results from a meta-analysis found the –1082G allele significantly increased the risk of digestive cancer.[39] Similarly, another meta-analysis indicated that *IL10* –1082 was associated with the risk of GC especially in Asian populations.[40]

The present study of 208 patients with GC, 116 patients with AG and 232 healthy control subjects, found no significant relationship between *IL10* –1082A/G and –592A/C polymorphisms and the risks of GC or AG in heterozygous, homozygous, dominant and recessive genetic models. A previous meta-analysis including 11 studies suggested a 13% reduced risk of GC conferred by the –819T allele compared with the –819C allele.[41] This meta-analysis indicated a protective role of the T allele of the *IL10* gene –819C/T polymorphism, which was in direct contrast to the results of this present research. In this present study, the CC genotype of the *IL10* gene –819C/T polymorphism was associated with a decreased risk in AG→GC compared with the TT genotype. In a recessive genetic model, a significant decreased risk of GC was found compared with AG and non-cancer subjects, respectively. The different results between this current study and the meta-analysis might be due to differences between the study populations, which will require further investigations conducted in multiple ethnicities to confirm. Another meta-analysis was performed on the *IL10* –592C>A polymorphism and GC.[42] No significant association was found between the *IL10* gene –592C>A polymorphism and GC risk in a total population analysis,[42] which was in accordance with the results of this current study.

Subgroup analysis in the current study suggested that in a recessive genetic model of the *IL10* gene –819C/T polymorphism, the CC genotype was significantly associated with a decreased risk of GC in males compared with AG and non-cancer, respectively. Generally, males have a higher risk of GC and relatively worse living habits compared with females, which might partly explain the detected significant association in males. As a well-known environmental pathogenic factor, chronic *H. pylori* infection could induce consistent inflammation.[43] Extensive evidence has revealed that chronic inflammation is involved in the initiation and development of GC.[44] As a result, *H. pylori* might be implicated in the relationship between polymorphisms of the inflammation-related gene *IL10* and the occurrence of AG and GC, and might exert a degree of interaction. In the subgroup analysis of the *H. pylori*-negative participants in the current study, the CC genotype also demonstrated a significant decreased GC risk. Furthermore, no

significant interaction was observed between the *IL10* gene polymorphisms and *H. pylori*. These results indicated that *H. pylori* might not be implicated in the effect of *IL10* gene polymorphisms on gastric carcinogenesis.

Interleukin-10 plays an essential role in coordinating local tissue inflammation and suppressing the immune response, thus it might exert an important effect in gastric carcinogenesis.[45] Because the IL-10-mediated inflammatory and immune environment might result in a change to the carcinogenic damage in cells, it might predispose an individual to develop GC. As a result, polymorphisms located in the promoter region of the *IL10* gene might influence the binding affinity of transcriptional factors and promoter activity, thereby altering *IL10* gene expression. In this current study, haplotype analysis suggested that the ACC haplotype was associated with a significantly decreased risk of GC compared with AG. The ATC haplotype was associated with a decreased risk of AG compared with the CON group, but was associated with an increased risk of GC compared with AG. Promoter polymorphisms of the *IL10* gene demonstrated their combined effect in regulating gene expression and disease risk. Further large-scale study is needed to confirm the relationship between *IL10* promoter polymorphisms and gastric carcinogenesis in various ethnicities.

In conclusion, the promoter −819C/T (rs1800871) polymorphism of the *IL10* gene was associated with the risk of GC and AG in a Chinese population. A consistent relationship was also found in males and *H. pylori*-negative subgroups. No significant interaction was detected between *IL10* promoter polymorphisms and *H. pylori*. In haplotype analysis, the ACC haplotype was associated with a significantly decreased risk of GC compared with AG. The ATC haplotype was associated with

a decreased risk of AG compared with the healthy controls, but was associated with an increased risk of GC compared with AG.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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