RESEARCH ARTICLE

Interleukin-4 and -8 Gene Polymorphisms and Risk of Gastric Cancer in a Population in Southwestern China

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Abstract

Background: Gastric carcinogenesis is a complicated process that involves environmental and genetic factors like interleukin-4 (IL-4) and IL-8. Single nucleotide polymorphisms in their genes are associated with changed levels of gene expression. Here, we investigated the association between IL4-590 C>T and IL8-251T>A and gastric cancer (GC) risk in Sichuan of Southwestern China. Materials and Methods: We surveyed the research subjects using a self-designed questionnaire with questions on demographic factors and putative risk factors. Approximately 2-5ml of whole blood was collected after field survey to analyze IL4-590 C>T and IL8-251T>A genotypes using MALDI-TOF MS. Results: Our study recruited 308 pairs of GC patients and controls, including 224 (72.7%) men and 84 (27.3%) women in each group. There were 99 cardia and 176 noncardia GC patients in the case group. The case and control groups had an average age of 57.7±10.6 (mean±SD) and 57.6±11.1 years. GC patients reported a significantly greater proportion of family history of cancer (29.9% *vs* 10.7%, $p<0.01$) and drinking (54.6% *vs* 43.2%, $p<0.01$) than did controls. Variant genotypes of IL-4-590 C>T and IL-8-251 T>A were not associated with overall GC risk (adjusted OR, 0.89; 95%CI, 0.61-1.28 for CT or CC *vs* TT; adjusted OR, 1.14; 95%CI, 0.86-1.79 for TA or AA *vs* TT). Stratification analysis of two SNPs for risk by subsites only found that variant IL-8-251 TA or AA genotype was associated with increased noncardia GC risk (adjusted OR, 2.58; 95%CI, 1.19-5.57). We did not observe interactions between the IL-8-251 T>A genotype and smoking (adjusted OR, 0.38; 95%CI, 0.08-1.79) or drinking (adjusted OR, 0.36; 95%CI, 0.08-1.65) for risk of noncardia GC. Conclusions: Our data indicate no association between the two SNPs of IL-4-590 and IL-8-251 with overall GC risk, while the IL-8-251 TA or AA genotype conferred risk of cardia GC. Our findings contribute to the evidence body for risk of SNPs associated with the development of gastric cancer in this region.

Keywords: Gastric cancer - interleukin-4 gene - interleukin-8 gene - polymorphism - risk - case-control study

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Introduction

According to the Chinese Ministry of Health data, cancer is implicated in approximately 26.33% of all deaths countrywide (Shen, 2011). It has already become the leading cause of death in urban areas, while it falls only behind cerebrovascular diseases as the second leading cause of death, accounting for 23.11% of deaths in rural areas. In particular, gastric cancer (GC) ranks as the third major malignancy in terms of mortality (Health, 2011). About 463,000 new cases of GC were estimated to have occurred in China in 2008, close to 46.9% of the worldwide estimate (988,000 cases) (Ferlay et al., 2010).

GCs are anatomically classified as noncardia and cardia types arising from different subsites, of which the former constitutes the majority of the cases. The two subtypes predominate in populations specific to different geographic locations, racial origins and socioeconomic backgrounds (Heidl et al., 1993a; 1993b; Crew et al., 2006). In face of the overall decrease in GC, increasing incidence of cardia subtype is reported in high-risk areas of China, more often in parallel with rise in esophageal cancer (He et al., 2008). Additionally, noncardia GC remains common in Chinese, as opposed to substantial declines in developed regions of the world (Kamangar et al., 2006). Two subtypes may vary in genetic susceptibility,

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pathogenesis, clinical presentations, and prognosis (Heidl et al., 1993a; 1993b; Crew et al., 2006). They are apparently distinguished by the findings that H. pylori infection is a major risk factor for the noncardia subtypee (Brenner et al., 2004; Kamangar et al., 2006), whereas gastroesophageal reflux disease is often involved in the development of cardia subtype (Lagergren et al., 1999).

Gastric carcinogenesis is a complicated process that involves environmental and genetic factors. Causes of GC remain not fully understood. Risk factors such as *Helicobacter pylori* (HP), salted/preserved food consumption, excessive alcohol intake, and tobacco smoking are implicated in the development of GC (Liu et al., 2008; Bertuccio et al., 2009; Gonzalez et al., 2012). Correspondingly, improvements in diet, food storage and a decline in

HP infection due to a general improvement in sanitary conditions have been thought to lead to worldwide decline of GC incidence in the last few decades (Thun et al., 2010).

Interleukin-4 (IL-4) and IL-8 are recognized as contributing factors to gastric carcinogenesis (Yuzhalin, 2011). They are dispensable components in the inflammation pathway which is regarded as an essential part of the carcinogenic process in GC (Macarthur et al., 2004). IL-4 has important functions in the tumor immunology, including the promotion of B cell growth, regulation of T cells, generation of cytotoxic effector cells, and growth and differentiation effects on hematopoetic cells (Crusius et al., 2008). It plays a key role in the maturation of T-helper cells to the Th2 phenotype; with a shift from a Th1 to Th2 cell pattern, it can enhance the production of anti-inflammatory cytokines and inhibit the production of monocyte-derived pro-inflammatory cytokines including IL-8 (Sugimoto et al., 2010). IL-8 enhances cell proliferation and migration, and acts like a chemoattractant for neutrophils and leukocytes, and a proangiogenic factor and mediator of chronic inflammatory processes (Kitadai et al., 1999). It is involved in adhesion, migration, and invasion in human GC cells *in vitro* (Ju et al., 2012) and highly expressed in GC cells (Xue et al., 2012), and increased IL-8 level may promote the inflammatory response that leads to risk of GC (Sugimoto et al., 2010).

Gene polymorphisms that modify the intensity of the inflammatory response may contribute to GC risk (Gonzalez et al., 2002). Single nucleotide polymorphisms (SNPs) of the IL-4 and IL-8 genes are potential biomarkers that predispose individuals to GC. The IL4 gene is located on chromosome 5q31-33. The C-to-T base substitution at position -590 (C-590T, rs2243250) of the IL4 gene promoter is associated with increased IL4 gene expression (Rosenwasser et al., 1995). The IL8 gene is located at chromosome 4q 13-q21. Of three common polymorphisms in the IL8 gene, the T-to-A base substitution at -251 (T-251A, rs4073) of the promoter region is associated with increased IL-8 production (Hull et al., 2000). To date, although multiple studies have been conducted on association of IL4-590 C>T (Loh et al., 2009; Sugimoto et al., 2010; Zhang et al., 2013) and IL8-251 T>A (Gianfagna et al., 2008; Gao et al., 2010; Liu et al., 2010; Wang et al., 2010; Persson et al., 2011; Yuzhalin, 2011; Wang et al., 2012; Xue et al., 2012) and GC among Asians, Caucasians and Hispanics, results are hardly conclusive with evident variability according to race. Even within the Chinese population, studies report conflicting results among studies in Taiwan (Wu et al., 2003; Lai et al., 2005; Lee et al., 2005), Fujian (Lin, 2008), Hebei (Zhang et al., 2010), Beijing (Lu et al., 2005), Guangdong and Shanxi (Zeng et al., 2005) for each polymorphism. Noticing the variability of results, we considered that it was necessitated to initiate a study on IL4-590 C>T and IL8-251T>A for risk of GC in the southwestern region of China.

In a 1:1 case-control study matched by age and sex, we investigated the association between IL4-590 C>T and IL8-251T>A, and GC risk in Sichuan of Southwestern China. Since etiologic factors for noncardia GC may differ from those for cardia subtype (Blaser, 1999; McColl et al., 2000; Brown et al., 2002), we additionally conducted subanalysis of the two SNPs for risk of two subtypes through stratification.

Materials and Methods

Subjects and survey

The study design was reported previously (Pan et al., 2013). In brief, patients with histologically confirmed GC were enrolled from Yanting Cancer Hospital and Institute, Sichuan University West China Hospital, and Sichuan Cancer Hospital between October 2010 and August 2011. Control subjects were selected from Sichuan University West China Fourth Hospital and a peri-urban community and matched at 1:1 to the cases by age (±3 years) and sex. Control subjects were not relatives of cases, and had no digestive diseases or a prior history of cancer. We surveyed the research subjects using a self-designed questionnaire with questions on demographic factors (e.g. age, sex, education background and marital status) and putative risk factors such as smoking, alcohol consumption, and family history of cancer. Smokers, drinkers, family history of cancer, and cardia and non-cardia cancers were defined in our previous study (Wen et al., 2012). The research was approved by the ethics committees of four hospitals, and informed consent was obtained from all recruited subjects.

DNA extraction and SNP genotyping

DNA extraction and SNP genotyping procedures were reported in prior studies (Pan et al., 2012; Wen et al., 2012; Pan et al., 2013). Approximately 2-5ml of whole blood was collected after field survey and stored at -20 °C. Genomic DNA was extracted within one week using the TIANamp blood DNA kit (Tiangen Biotech, Beijing, China) as per the manufacturer's instructions. SNP genotyping was performed in a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). Primers for polymerase chain reaction amplification and single base extension (SBE) assays were designed using Sequenom Assay Design 3.1 software (Table 1). IL4-590 C>T and IL8-251T>A genotypes were analyzed using MALDI-TOF MS according to Justenhoven et al. (Justenhoven et al., 2004), and procedures were explained in our previous study (Wen et al., 2012). The MassARRAY Analyzer Compact with ACQUAIRE Module (Sequenom)

acquired spectra from the SpectroCHIP, which were automatically processed and saved to the MassARRAY database.

Statistical analysis

Statistical analysis was performed using Stata 8.0 (StataCorp, College Station, USA). Continuous variables were expressed as mean±standard deviation (SD), while categorical variables were presented as frequencies and percentages. Demographic characteristics were compared between cases and controls by means of McNemar test. Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of IL4-590 C>T and IL8-251T>A genotypes among control group using the Chi-square test. Conditional logistic regression was used to calculate odd ratios (OR) and 95% confidence intervals (CIs). All P values were two-tailed and statistical significance was indicated by a value of $p<0.05$.

Results

Demographic characteristics of research subjects

Our study recruited 308 pairs of GC patients and controls, including 224 (72.7%) men and 84 (27.3%) women in each group. The case and control groups had an average age of 57.7±10.6 (mean±SD) and 57.6±11.1 years, respectively. General demographic characteristics of research subjects were reported in a previous study (Pan et al., 2013), and are reproduced in Table 2. In general, GC patients reported a significantly greater proportion of family history of cancer than did controls (29.9% versus 10.7%, $p<0.01$). More drinkers were represented in cases compared with controls ($p<0.01$). No statistically significant difference was noted between cases and controls in terms of education and smoking status ($p=0.68$ and 0.74). Information of 99 cardia and 176 noncardia GC patients and controls is also summarized in Table 2, while cases with undefined subsite and matched controls are excluded from analysis. Similar results were obtained by subsite of occurrence, though drinking was not more prevalent in cardia cancer patients than controls ($p=0.39$).

Genotype distributions and their association with overall GC

The genotype distributions of IL-4-590 C>T and IL-8-251 T>A, and their association with overall GC risk are presented in Table 3. The genotype distributions of two polymorphisms in controls were in agreement with those predicted under the conditions of Hardy-Weinberg equilibrium ($\chi^2=0.74$, $p=0.39$; $\chi^2=0.13$, $p=0.72$). Genotyping results demonstrated that the allele frequencies for IL-4-590 T and IL-8-251 A respectively were 80.52% and 42.86% in cases and 80.78% and 43.18% in controls. The allele frequencies of IL-4-590 C>T and IL-8-251 T>A among the cases were not statistically significantly different from those among controls ($\chi^2=0.01$, $p=0.91$; $\chi^2=0.61$, $p=0.43$). Multivariate regression analyses showed that subjects carrying the IL-4-590 T or IL-8-251A variant allele had non-significant decreased risk of GC. Noticing the prevalence of the variant genotype, IL-4-590 TT, we combined the wild-type and variant heterozygous genotypes for analysis, and found that subjects carrying IL-4-590 CT or CC genotype showed borderline decreased risk for developing GC (adjusted OR, 0.89; 95%CI, 0.61-1.28) after adjusting for family history of cancer, drinking, and smoking. Because of the low allele frequencies and relative rarity of the homozygous variant genotype, IL-8-251 AA, we combined the homozygous variant and heterozygous groups for analysis of IL-8-251 T>A. The IL-8-251 T>A variant genotype was not

Table 1. Primer Sequences and Masses for IL-4-590 C>T and IL-8-251 T>A

	IL-4-590 C>T	IL-8-251 T>A
Primer sequence (5'-3')	GAATAACAGGCAGACTCTCC CGACCTGTCCTTCTCAAAAC	GCCACTCTAGTACTATATCTG CTGAAGCTCCACAATTTGGT
Unextended primer sequence	CTACCCCAGCACTGGGG	GTTATCTAGAAATAAAAAAGCATACA
Mass_UEX[a] (Da)	5156.4	8003.3
Variant alleles	C/T	A/T
Mass_EX[b] (Da)	5427.6/5443.6	8274.5/8330.4

*[a] Mass_UEX indicates mass of un-extended primer (Da); [b] Mass_EX indicates mass of extended primer (Da)

Table 2. Characteristics of GC and Control Subjects

Variables, n (%)	Overall (n=308)			Cardia (n=99)			Noncardia (n=176)		
	Cases	Controls	*p* value[a]	Cases	Controls	*p* value[a]	Cases	Controls	*p* value[a]
Education									
Primary or below	139 (45.42)	144 (46.75)	0.68	61 (61.62)	50 (50.51)	0.57	63 (36.21)	79 (44.89)	0.35
Secondary	93 (30.39)	93 (30.19)		27 (27.27)	31 (31.31)		58 (33.33)	55 (31.25)	
High or technical school	48 (15.69)	55 (17.86)		8 (8.08)	14 (14.14)		35 (20.11)	30 (17.05)	
College or above	26 (8.50)	16 (5.91)		3 (3.03)	4 (4.04)		18 (10.34)	12 (6.82)	
Family history of cancer									
No	216 (70.13)	275 (89.29)	<0.01	65 (65.66)	85 (85.86)	<0.01	131 (74.43)	161 (91.48)	<0.01
Yes	92 (29.87)	33 (10.71)		34 (34.34)	14 (14.14)		45 (25.57)	15 (8.52)	
Smoking									
Non-smokers	128 (41.56)	132 (42.86)	0.74	38 (38.38)	37 (37.37)	0.99	77 (43.75)	83 (47.16)	0.30
Smokers	180 (58.44)	176 (57.14)		61 (61.62)	62 (62.63)		99 (56.25)	93 (52.84)	
Drinking									
Non-drinkers	140 (45.45)	175 (56.82)	<0.01	42 (42.42)	51 (51.52)	0.39	81 (46.02)	102 (57.95)	<0.01
Drinkers	168 (54.55)	133 (43.18)		57 (57.58)	48 (48.48)		95 (53.98)	74 (42.05)	

*[a] *p* value by the McNemar test

associated with risk of GC after adjusting for confounding variables (adjusted OR, 1.14; 95%CI, 0.86-1.79).

Subanalysis of genotype distributions and their association with cardia and noncardia GC

The genotype distributions of IL-4-590 C>T and IL-8-251 T>A and their association with cardia and noncardia GC are presented in Table 4. The genotype distributions of two polymorphisms in matched controls for two subtypes conformed to the Hardy-Weinberg equilibrium (χ^2=0.09, p=0.76 and χ^2=1.64, p=0.20 for the IL-4 polymorphism; χ^2=1.97, p=0.16 and χ^2=0.75, p=0.39 for the IL-8 polymorphism). Genotyping results demonstrated that the allele frequencies for IL-4-590 T and IL-8-251 A, respectively, were 80.81% and 45.96% in cardia cases and 83.84% and 44.44% in controls. The allele frequencies of IL-4-590 C>T and IL-8-251 T>A were not statistically significantly different from those in controls (χ^2=0.63, p=0.43; χ^2=0.09, p=0.76). Multivariate regression analyses showed that subjects carrying the IL-4-590 T variant allele had a non-significant decreased risk. The distribution of IL-8-251 T>A were statistically significant between the cases and controls (χ^2 =4.26, p=0.04). The variant IL-8-251 TA or AA genotype was associated with increased noncardia GC risk (adjusted OR, 2.58; 95%CI, 1.19-5.57). We analyzed the interaction between IL-8-251 T>A genotype and smoking or drinking for risk of cardia GC. In a multiplicative model, we did not observe interaction between the IL-8-251 T>A genotype and smoking (adjusted OR, 0.38; 95%CI, 0.08-1.79) or drinking (adjusted OR, 0.36; 95%CI, 0.08-1.65) for risk of noncardia GC.

Table 3. IL-4-590 C>T and IL-8-251 T>A Genotypes and Alleles with Gastric Cancer Risk

SNP	Cases (n=308)	Controls (n=308)	OR (95%CI)[a] Crude	Adjusted[b]
IL-4-590 C>T[c]				
TT	200 (64.94)	198 (64.50)	1 (reference)	1 (reference)
CT+CC	108 (35.07)	109 (35.50)	0.99 (0.70-1.39)	0.89 (0.61-1.28)
T allele	496 (80.52)	496 (80.78)	1 (reference)	
C allele	120 (19.48)	118 (19.22)	1.02 (0.77-1.35)	
IL-8-251 T>A				
TT	92 (29.87)	101 (32.79)	1 (reference)	1 (reference)
AT+AA	216 (70.13)	207 (67.21)	1.14 (0.82-1.59)	1.14 (0.86-1.79)
T allele	352 (57.14)	350 (56.82)	1 (reference)	
A allele	264 (42.86)	266 (43.18)	0.99 (0.79-1.24)	

[a]ORs and 95%CIs were calculated by conditional logistic regression. b ORs were adjusted for family history of cancer, drinking, and smoking. c IL4-590 C>T was not detected in one control subject

We found that the allele frequencies for IL-4-590 T and IL-8-251 A, respectively, were 81.53% and 41.76% in noncardia cases and 78.86% and 43.18% in controls. The allele frequencies of IL-4-590 C>T and IL-8-251 T>A were not statistically significantly different from those in controls (χ^2=0.79, p=0.37; χ^2=0.15, p=0.70). Multivariate regression analyses showed that subjects carrying the IL-4-590 T or IL-8-251 A variant allele were not associated with risk of noncardia GC. The IL-4-590 CT or CC genotype, and IL-8-251 TA or AA genotype were associated with nonsignificant protective effect against noncardia GC risk (adjusted OR, 0.66; 95%CI, 0.39-1.09; adjusted OR, 0.89; 95%CI, 0.56-1.41).

Discussion

The association between inflammation and cancer is well established, and in particular GC is a prime example, in which HP infection induces chronic gastric inflammation that progresses to atrophy, metaplasia, dysplasia, and malignancy (Fox et al., 2007).

Since inflammation is recognized as a contributing factor in the pathogenesis of many cancers, the search for genetic factors that predispose to GC has capitalized on pro-inflammatory and anti-inflammatory cytokines. Given that individual genetic differences caused by SNPs may eventually play a role in gastric carcinogenesis, we conducted a series of inflammation-related SNP analyses for risk of GC, and findings so far have shown that IL-10-592 A>C (Pan et al., 2013)was associated with noncardia GC subsites in a southwestern region of China. In this study, we aimed to determine whether IL-4-590 C>T and IL-8-251 T>A contributed to risk of overall GC and two different subtypes. To our knowledge, our study is the first that investigates these two polymorphisms in the development of GC in this region. An important finding was that both polymorphisms were not associated with overall GC risk and IL-8-251 T>A possibly contributed to cardia GC in subanalysis.

In our study, no association was detected between IL-8-251 T>A polymorphism and overall GC. Our result for the overall GC risk was inconsistent with that from two other studies in Eastern and Southeastern China (Zeng et al., 2005), Northern China (Lu et al., 2005), and Chinese Taiwan (Lee et al., 2005); in former two studies, AA was suggested as a hazard genotype for GC, while TT rendered a risk in the Taiwan study. This indicates possible

Table 4. IL-4-590 C>T and IL-8-251 T>A Genotypes and Alleles with Cardia and Noncardia GC Risk

SNP	cardia (n=99)				noncardia (n=176)[c]			
	Cases	Controls	cOR (95%CI)[a]	aOR (95%CI)[b]	Cases	Controls	cOR (95%CI)[a]	aOR (95%CI)[b]
IL-4-590 C>T								
TT	63 (63.64)	70 (70.71)	1 (reference)	1 (reference)	118 (67.05)	106 (60.57)	1 (reference)	1 (reference)
CT+CC	36 (36.36)	29 (29.29)	1.37 (0.76-2.47)	1.28 (0.68-2.43)	58 (32.95)	69 (39.43)	0.73 (0.46-1.17)	0.66 (0.39-1.09)
T allele	160 (80.81)	166 (83.84)	1 (reference)		287 (81.53)	276 (78.86)	1 (reference)	
C allele	38 (19.19)	32 (16.16)	1.23 (0.73-2.07)		65 (18.47)	74 (21.14)	0.85 (0.58-1.23)	
IL-8-251 T>A								
TT	21 (21.21)	34 (34.34)	1 (reference)	1(reference)	59 (33.52)	54 (30.68)	1 (reference)	1 (reference)
AT+AA	78 (78.79)	65 (65.66)	2.08 (1.05-4.15)	2.58 (1.19-5.57)	117 (66.48)	122 (69.32)	0.89 (0.59-1.36)	0.89 (0.56-1.41)
T allele	107 (54.04)	110 (55.56)	1 (reference)		205 (58.24)	200 (56.82)	1 (reference)	
A allele	91 (45.96)	88 (44.44)	1.06 (0.72-1.58)		147 (41.76)	152 (43.18)	0.94 (0.70-1.27)	

[a]ORs and 95%CIs were calculated by conditional logistic regression and cOR stands for crude odds ratio. b ORs were adjusted for family history of cancer, drinking, and smoking and aOR stands for adjusted odds ratio. c IL4-590 C>T was not detected in one control subject

heterogeneity of results even within a country owing to geographic, environmental and sociodemographic diversity in different populations (Pan et al., 2013). In addition, these studies adjusted different confounding factors (Lee et al., 2005) or apparently ignored potential confounding from lifestyle or environmental factors for overall analysis (Lu et al., 2005; Zeng et al., 2005), so the results might not be comparable to some extent. Results from studies among other races were also mixed. In particular, there was null result for overall GC risk among European populations (Kamangar et al., 2006; Canedo et al., 2008; Crusius et al., 2008), whereas positive association was observed in Korea (Kang et al., 2009) and Japan (Taguchi et al., 2005). In an effort to isolate the contribution of the cytokine genotype to different subtypes of GC, stratification analysis was also performed according to subsites (cardia versus noncardia) in our study. We found that the TA or AA genotype had a significant effect on the risk of cardia GC, which agrees well with the finding from Northern China (Zhang et al., 2010) and is corroborated by a meta-analysis that pooled several studies (Wang et al., 2010). The risk was even more apparent in HP positive individuals in that study when the population was stratified, which signals that HP infection as an important pathogen that initiates inflammation may confound the correlation between a SNP and GC risk and thus should be controlled for SNP analysis. On the contrast, the positive association with the IL-8-251 TA or AA genotype was only restricted to noncardia GC risk in Chinese Taiwan and Korea (Lee et al., 2005; Ye et al., 2009), while in a European multi-country study, the A allel was associated with a significant reduced risk of noncardia GC (OR 0.57; 95%CI 0.37-0.87) that was restricted to the Hp-positive group (Crusius et al., 2008). Inconsistent or even contradictory findings from different GC association studies reflect variable genetic background such as IL-8-251 A allele frequency and impact of lifestyles or environment among difference races (Wang et al., 2010), status of HP infection, and heterogeneity of GC phenotypes such as different modes of pathogenesis (Schneider et al., 2008). It is hypothesized that the IL8-251 A allele may be in linkage disequilibrium with an unidentified sequence variant that correlates with GC development. It is thus logical to observe distinct associations in different populations since haplotype structure may vary considerably between distinct populations (Canedo et al., 2008). To clarify whether smoking and drinking modified the risk of cardia GC posed by the variant genotype, we tentatively analyzed the interaction of the IL IL-8-251 T>A polymorphism and smoking or drinking, but we could not identify synergic effects. Unfortunately, we were not able to stratify the cases and controls by HP infection status for subanalysis or controlling for its effect. This may prevent us from extrapolating our results to a wider context. However, as in established paradigm HP infection is not a risk factor for cardia GC, the positive association identified is thus not unfounded in our study though the statistical power is limited due to a small sample size (99 cases of cardia GC). In future studies, haplotype based approaches that involve genotyping of several genetic markers may be adopted to efficiently capture the genetic diversity and clarify the association between the IL-8-251 T>A polymorphisms and GC risk in different populations (Canedo et al., 2008).

The IL-4-590 C>T polymorphism was not associated with either overall or subtype GC. This finding concerning overall GC risk was in agreement with that from studies in Chinese Taiwan (Wu et al., 2003; Lai et al., 2005), Spain (Garcia-Gonzalez et al., 2007), and Venezuela (Kato et al., 2006). Of note, the risk of the polymorphism remained nonsignificant even in a case-control analysis for HP infected patients and controls in a Japanese population (Ando et al., 2009). We observed that T was a dominant allele in Asian populations (Wu et al., 2003; Lai et al., 2005; Ando et al., 2009), but a minor one in European and African populations (Kato et al., 2006; Garcia-Gonzalez et al., 2007). Despite distinct allele and genotype frequencies, the negative association with GC is, however, consistent among different populations. Our finding is also validated in a meta-analysis that pooled all existing studies for association analysis for cancer risk and subgroup analysis for GC (Zhang et al., 2013). What is noteworthy is that a higher risk of developing diffuse type (OR, 1.64; 95%, CI 1.01-2.67) or cardia cancer (OR, 2.44, 95%CI 1.13-2.67) was observed for the CT/CC genotype in Chinese Taiwan, which is contradictory to our result for subgroup analysis (Wu et al., 2003). Given contrasting nonsignificant effects existed for cardia (OR, 1.28; 95%CI, 0.68-2.43) and noncardia GC (OR, 0.66; 95%CI, 0.39-1.09) during subanalysis in our study, we suspect that the negative association for overall GC risk in all studies are due to counterbalancing effect of the polymorphism for two different subtypes. In addition, if a larger sample was available, statistical significance might be achieved for risk of cardia GC.

There are certain limitations in this study. Firstly, HP infection was not detected and adjusted for studying the inflammation related polymorphisms. This is an evident downside in this study and needs attention in future designed studies. Secondly, the two polymorphisms were independently analyzed for risk of GC without considerations of the effect of other polymorphisms of pro-inflammatory and anti-inflammatory cytokines such as IL-2, IL-10, IL-1B and IL-1RN. Haplotype analysis of different functional polymorphisms relating to inflammation in a large population might be feasible to account for potential linkage disequilibrium existing between them. Thirdly, clinical staging and histological types were not given consideration in our analysis, since such data were lacking. The diffuse-type GC has a different pathological carcinogenesis mechanism from that of the intestinal subtype which is usually preceded by gastric precursor lesions such as gastric atrophy and intestinal metaplasia (Camargo et al., 2006). It is also likely that the inflammation related cytokines may have changing roles at different stages of GC that may contradict with each other. Screening for applicable SNPs for either functional analysis or clinical practice may lose its utility without these considerations.

In conclusion, IL-4-590 C>T and IL-8-251 T>A were not associated with overall GC risk in Western China, while the IL-8-251 TA or AA genotype conferred risk of

cardia GC. Future studies will be needed to validate our finding in this region and explore other SNPs that can be utilized in conjunction with other risk assessment for clinical screening of high-risk individuals.

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References

Ando T, Ishikawa T, Kato H, et al (2009). Synergistic effect of HLA class II loci and cytokine gene polymorphisms on the risk of gastric cancer in Japanese patients with *Helicobacter pylori* infection. *Int J Cancer*, **125**, 2595-602.

Bertuccio P, Chatenoud L, Levi F, et al (2009). Recent patterns in gastric cancer: a global overview. *Int J Cancer*, **125**, 666-73.

Blaser MJ (1999). Hypothesis: the changing relationships of *Helicobacter pylori* and humans: implications for health and disease. *J Infect Dis*, **179**, 1523-30.

Brenner H, Arndt V, Stegmaier C, et al (2004). Is *Helicobacter pylori* infection a necessary condition for noncardia gastric cancer? *Am J Epidemiol*, **159**, 252-8.

Brown LM, Devesa SS (2002). Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am*, **11**, 235-56.

Camargo MC, Mera R, Correa P, et al (2006). Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*, **15**, 1674-87.

Canedo P, Castanheira-Vale AJ, Lunet N, et al (2008). The interleukin-8-251*T/*A polymorphism is not associated with risk for gastric carcinoma development in a Portuguese population. *Eur J Cancer Prev*, **17**, 28-32.

Crew KD, Neugut AI (2006). Epidemiology of gastric cancer. *World J Gastroenterol*, **12**, 354-62.

Crusius JBA, Canzian F, Capella G, et al (2008). Cytokine gene polymorphisms and the risk of adenocarcinoma of the stomach in the European prospective investigation into cancer and nutrition (EPIC-EURGAST). *Ann Oncol*, **19**, 1894-902.

Ferlay J, Shin HR, Bray F, et al (2010). GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet], Lyon, France: International Agency for Research on Cancer; 2010.

Fox JG, Wang TC (2007). Inflammation, atrophy, and gastric cancer. *J Clin Invest*, **117**, 60-9.

Gao LB, Pan XM, Jia J, et al (2010). IL-8 -251A/T polymorphism is associated with decreased cancer risk among population-based studies: evidence from a meta-analysis. *Eur J Cancer*, **46**, 1333-43.

Garcia-Gonzalez MA, Lanas A, Quintero E, et al (2007). Gastric cancer susceptibility is not linked to pro-and anti-inflammatory cytokine gene polymorphisms in whites: A nationwide multicenter study in Spain. *Am J Gastroenterol*, **102**, 1878-92.

Gianfagna F, De Feo E, Van Duijn CM, et al (2008). A systematic review of meta-analyses on gene polymorphisms and gastric cancer risk. *Curr Genomics*, **9**, 361-74.

Gonzalez CA, Agudo A (2012). Carcinogenesis, prevention and early detection of gastric cancer: where we are and where we should go. *Int J Cancer*, **130**, 745-53.

Gonzalez CA, Sala N, Capella G (2002). Genetic susceptibility and gastric cancer risk. *Int J Cancer*, **100**, 249-60.

He YT, Hou J, Chen ZF, et al (2008). Trends in incidence of esophageal and gastric cardia cancer in high-risk areas in China. *Eur J Cancer Prev*, **17**, 71-6.

Health CMO (2011). China Health Statistics Yearbook - 2010, Beijing:Peking Union Medical College Press, 2010.

Heidl G, Langhans P, Krieg V, et al (1993). Comparative studies of cardia carcinoma and infracardial gastric carcinoma. *J Cancer Res Clin Oncol*, **120**, 91-4.

Heidl G, Langhans P, Mellin W, et al (1993). Adenocarcinomas of esophagus and cardia in comparison with gastric carcinoma. *J Cancer Res Clin Oncol*, **120**, 95-9.

Hull J, Thomson A, Kwiatkowski D (2000). Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax*, **55**, 1023-7.

Ju D, Sun D, Xiu L, et al (2012). Interleukin-8 is associated with adhesion, migration and invasion in human gastric cancer SCG-7901 cells. *Med Oncol*, **29**, 91-9.

Justenhoven C, Hamann U, Pesch B, et al (2004). ERCC2 genotypes and a corresponding haplotype are linked with breast cancer risk in a German population. *Cancer Epidem Biomar*, **13**, 2059-64.

Kamangar F, Abnet CC, Hutchinson AA, et al (2006). Polymorphisms in inflammation-related genes and risk of gastric cancer (Finland). *Cancer Causes Control*, **17**, 117-25.

Kamangar F, Dawsey SM, Blaser MJ, et al (2006). Opposing risks of gastric cardia and noncardia gastric adenocarcinomas associated with *Helicobacter pylori* seropositivity. *J Natl Cancer Inst*, **98**, 1445-52.

Kamangar F, Dores GM, Anderson WF (2006). Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol*, **24**, 2137-50.

Kang JM, Kim N, Lee DH, et al (2009). The effects of genetic polymorphisms of IL-6, IL-8, and IL-10 on Helicobacter pylori-induced gastroduodenal diseases in Korea. *J Clin Gastroenterol*, **43**, 420-8.

Kato I, Canzian F, Franceschi S, et al (2006). Genetic polymorphisms in anti-inflammatory cytokine signaling and the prevalence of gastric precancerous lesions in Venezuela. *Cancer Causes Control*, **17**, 1183-91.

Kitadai Y, Takahashi Y, Haruma K, et al (1999). Transfection of interleukin-8 increases angiogenesis and tumorigenesis of human gastric carcinoma cells in nude mice. *Br J Cancer*, **81**, 647-53.

Lagergren J, Bergstrom R, Lindgren A, Nyren O (1999). Symptomatic gastroesophageal reflux as a risk factor for esophageal adenocarcinoma. *N Engl J Med*, **340**, 825-31.

Lai KC, Chen WC, Jeng LB, et al (2005). Association of genetic polymorphisms of MK, IL-4, p16, p21, p53 genes and human gastric cancer in Taiwan. *Eur J Surg Oncol*, **31**, 1135-40.

Lee WP, Tai DI, Lan KH, et al (2005). The -251T allele of the interleukin-8 promoter is associated with increased risk of gastric carcinoma featuring diffuse-type histopathology in Chinese population. *Clin Cancer Res*, **11**, 6431-41.

Lin XH (2008). Correlation of interleukin 4-590 and intron 3 polymorphisms to genetic susceptibility to gastric cardiac carcinoma and noncardiac gastric carcinoma (Chinese).

Liu C, Russell RM (2008). Nutrition and gastric cancer risk: an update. *Nutr Rev*, **66**, 237-49.

Liu L, Zhuang W, Wang C, et al (2010). Interleukin-8 -251 A/T gene polymorphism and gastric cancer susceptibility: a meta-analysis of epidemiological studies. *Cytokine*, **50**, 328-34.

Loh M, Koh KX, Yeo BH, et al (2009). Meta-analysis of genetic polymorphisms and gastric cancer risk: variability in associations according to race. *Eur J Cancer*, **45**, 2562-8.

Lu W, Pan K, Zhang L, et al (2005). Genetic polymorphisms of interleukin (IL)-1B, IL-1RN, IL-8, IL-10 and tumor necrosis factor {alpha} and risk of gastric cancer in a Chinese population. *Carcinogenesis*, **26**, 631-6.

Macarthur M, Hold GL, El-Omar EM (2004). Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. *Am J Physiol Gastrointest Liver Physiol*, **286**, 515-20.

Mccoll KE, El-Omar E, Gillen D (2000). *Helicobacter pylori* gastritis and gastric physiology. *Gastroenterol Clin North Am*, **29**, 687-703.

Pan XF, Xie Y, Loh M, et al (2012). Polymorphisms of XRCC1 and ADPRT genes and risk of noncardia gastric cancer in a Chinese population: a case-control study. *Asian Pac J Cancer Prev*, **13**, 5637-42.

Pan XF, Yang SJ, Loh M, et al (2013). Interleukin-10 gene promoter polymorphisms and risk of gastric cancer in a Chinese population: single nucleotide and haplotype analyses. *Asian Pac J Cancer Prev*, **14**, 2577-82.

Persson C, Canedo P, Machado JC, et al (2011). Polymorphisms in inflammatory response genes and their association with gastric cancer: A HuGE systematic review and meta-analyses. *Am J Epidemiol*, **173**, 259-70.

Rosenwasser LJ, Klemm DJ, Dresback JK, et al (1995). Promoter polymorphisms in the chromosome 5 gene cluster in asthma and atopy. *Clin Exp Allergy*, **25**, 74-8.

Schneider BG, Camargo MC, Ryckman KK, et al (2008). Cytokine polymorphisms and gastric cancer risk: an evolving view. *Cancer Biol Ther*, **7**, 157-62.

Shen LY (2011). China Statisticl yearbook, Beijing: China Statistics Press, 2008.

Sugimoto M, Yamaoka Y, Furuta T (2010). Influence of interleukin polymorphisms on development of gastric cancer and peptic ulcer. *World J Gastroenterol*, **16**, 1188-200.

Taguchi A, Ohmiya N, Shirai K, et al (2005). Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. *Cancer Epidemiol Biomarkers Prev*, **14**, 2487-93.

Thun MJ, Delancey JO, Center MM, et al (2010). The global burden of cancer: priorities for prevention. *Carcinogenesis*, **31**, 100-10.

Wang J, Pan HF, Hu YT, et al (2010). Polymorphism of IL-8 in 251 allele and gastric cancer susceptibility: a meta-analysis. *Dig Dis Sci*, **55**, 1818-23.

Wang N, Zhou R, Wang C, et al (2012). -251 T/A polymorphism of the interleukin-8 gene and cancer risk: a HuGE review and meta-analysis based on 42 case-control studies. *Mol Biol Rep*, **39**, 2831-41.

Wen YY, Pan XF, Loh M, et al (2012). ADPRT Val762Ala and XRCC1 Arg194Trp polymorphisms and risk of gastric cancer in Sichuan of China. *Asian Pac J Cancer Prev*, **13**, 2139-44.

Wu MS, Wu CY, Chen CJ, et al (2003). Interleukin-10 genotypes associate with the risk of gastric carcinoma in Taiwanese Chinese. *Int J Cancer*, **104**, 617-23.

Xue H, Liu J, Lin B, et al (2012). A meta-analysis of interleukin-8 -251 promoter polymorphism associated with gastric cancer risk. *Plos One*, **7**, 28083.

Ye BD, Kim SG, Park JH, et al (2009). The interleukin-8-251 A allele is associated with increased risk of noncardia gastric adenocarcinoma in *Helicobacter pylori*-infected Koreans. *J Clin Gastroenterol*, **43**, 233-9.

Yuzhalin A (2011). The role of interleukin DNA polymorphisms in gastric cancer. *Hum Immunol*, **72**, 1128-36.

Zeng ZR, Zhou SZ, Liao SY, et al (2005). Correlation of polymorphism of interleukin 8 gene-251 locus and gastric cancer in high and low prevalence regions in China (Chinese). *J Sun Yat-sen Univ (Med Sci)*, 537-40.

Zhang J, Xie D, Zhou H, et al (2013). The -590C/T polymorphism in the IL-4 gene and the risk of cancer: a meta-analysis. *Tumour Biol*, **34**, 2261-8.

Zhang L, Du C, Guo X, et al (2010). Interleukin-8-251A/T polymorphism and *Helicobacter pylori* infection influence risk for the development of gastric cardiac adenocarcinoma in a high-incidence area of China. *Mol Biol Rep*, **37**, 3983-9.