RESEARCH ARTICLE

Interleukin-10 Gene Promoter Polymorphisms and Risk of Gastric Cancer in a Chinese Population: Single Nucleotide and Haplotype Analyses

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Abstract

Objectives: Interleukin (IL) -10 is a potent cytokine with a dual ability to immunosuppress or immunostimulate. We aimed to explore the association of *IL10* promoter polymorphisms with risk of gastric cancer (GC) in a Han population in Southwestern China. Methods: We enrolled 308 pairs of GC and control subjects from four hospitals and a community between October 2010 and August 2011 in a 1:1 matched case-control design. Demographic information was collected using a designed questionnaire. *IL10*-592 A>C and *IL10*-1082 A>G polymorphisms were determined by Sequenom MassARRAY analysis. Results: Patients with GC reported statistically higher proportions of family history of cancer (29.9% versus 10.7%, *P*<0.01) and alcohol drinking (54.6% versus 43.2%, *P*<0.01) than did controls. Similar results were observed in comparison between non-cardia GC patients and controls (*P*<0.01 and *P*=0.03). Variant genotypes of *IL10*-592 A>C and *IL10*-1082 A>G were not associated with overall GC risk (adjusted OR, 0.94, 95% CI, 0.66-1.33; adjusted OR, 1.00, 95% CI, 0.62-1.60). Sub-analysis showed that the *IL10*-592 AC/CC variant genotype was associated with decreased non-cardia GC risk (adjusted OR, 0.58; 95% CI, 0.36-0.95). No association was found between any of the *IL10* haplotypes established from two polymorphisms and risk of non-cardia GC. Conclusions: In conclusion, our data do not link the two SNPs of *IL10*-592 and *IL10*-1082 with overall GC risk. We demonstrate that *IL10*-592 polymorphism is associated with protective effect against non-cardia GC. Our findings may offer insight into risk associated with the development of GC in this region.

Keywords: Gastric cancer - interleukin-10 gene - polymorphism - risk - case-control study

Introduction

Gastric cancer (GC) is the fourth most common cancer worldwide with estimated 988,000 new cases and 736,000 deaths each year (Ferlay et al., 2008). Corresponding estimates for China are 464,400 new cases (47.0%) and 352,300 deaths (47.9%) (Ferlay et al., 2008). The age-standardized GC incidence rate in China is 16.5 per 100,000, 15% higher than the world average, 14.0 per 100,000 (Ferlay et al., 2008). In recent years, China has witnessed a rapid decrease in both incidence and mortality of GC, albeit at a lower magnitude than in western countries (Bertuccio et al., 2009). Despite the overall decrease in GC incidence, an increase has been observed in the oldest and youngest groups, and a less remarkable decline has been observed in women than men (Kamangar et al., 2006; Bertuccio et al., 2009). What

is noteworthy is that the age of onset of developing GC seems younger in China than in western countries. These substantial statistics and trends render GC as one of the priorities in cancer prevention and control in China.

Reasons for reductions in overall GC incidence and mortality have not been fully elucidated, but changes in lifestyle/environmental factors and improved health care could be possible factors. These include decreased intake of salted and preserved foods due to use of fridges, increased consumption of fruits and vegetables, reduced chronic Helicobacter pylori infection owing to better hygiene and medication, mass screening measures to detect precancerous lesions in some regions such as Japan, and decreased smoking in developed countries (Bertuccio et al., 2009; Jemal et al., 2010). However, since there is an estimated 15% difference in the age-standardized GC incidence between China and the world (Ferlay et al.,

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2008), there is much room for improvement and progress in GC control in China.

Carcinogenesis is a multfactorial process in which both genetic and environmental factors and their interactions are implicated, although the extent of respective contribution remains controversial. Particularly, the pathogenesis of GC represents a prototype of environment-gene interaction. Environmental exposure and lifestyle preferences have been studied in depth so far for association with GC. Individual variations in GC risk have been associated with specific polymorphisms of different genes that are present in a significant proportion of the normal population. Genetic polymorphisms may clarify the causes and events involved in gastric carcinogenesis. A variety of genes may be associated with gastric carcinogenesis, of which interleukin (IL) genes may be involved in occurrence and development of GC and therefore be applied as specific markers of predisposition for GC prevention (Yuzhalin, 2011).

The ILs represent a diverse constellation of cytokines that are small cell signaling protein molecules which regulate the function of the immune system in human. They are produced predominantly by T cells, monocytes, macrophages, and endothelial cells. They have multiple functions including facilitating communication between immune cells, controlling genes, regulating transcription factors, and governing the inflammation, differentiation, proliferation, and secretion of antibodies (Salazar-Onfray et al., 2007). Their coordinated work ensures the correct and effective functioning of immune system. Dysregulation between IL interactions or disruptions in the JAK (Janus-associated kinases) / STAT (signal transducers and activators of transcription) pathway may lead to DNA damage, excessive production of tumor-inducing factors, immune disorders, angiogenesis, and dysplasia (Yuzhalin 2011). Dysregulated cytokine signaling has also been linked to malignant transformation and further formation of metastasis (Salazar-Onfray et al., 2007). Genetic factors such as polymorphisms play an essential role in ILs balance, and single nucleotide polymorphisms (SNPs) of genes encoding ILs and their receptor may alter cytokine function and dysregulate its expression, as well as cause defects in cytokine cascades (Yuzhalin, 2011). Consequently, individual genetic differences caused by SNPs may be closely related to these disruptions and eventually play a role in gastric carcinogenesis.

Over 40 ILs are identified in human body (Akdis et al., 2011). IL-10 generally functions as an immunosuppressor and anti-inflammatory mediator that is known to have both anti-angiogenic and immunosuppressive properties (Wang et al., 2012). The gene encoding IL-10 is located on chromosome 1 (1q31-1q32). Three functional promoter SNPs in the *IL10* locus at -1082 (A to G, rs1800896), -819 (C to T, rs1800871), and -592 (A to C, rs1800872) from the transcriptional start site have been confirmed (Zhuang et al., 2010). In a meta-analysis in 2009, *IL10*-592 A>C and *IL10*-1082 A>G were moderately associated with GC risk in Asians, whereas no such association was noted among Caucasians (Loh et al., 2009). *IL10*-819 C>T was consistently not significant for GC risk in Asians or Caucasians. There seems to be considerable variability of the risk of GC associated with these polymorphisms according to races. In addition, many of the included Chinese studies were conducted in Chinese Taiwan and Northern China. Given the environmental and genetic regional nuances within China, we hypothesized that these polymorphisms may confer a different level of risk of GC in Southwestern China.

In the current article, we describe a 1:1 case–control study matched by age and sex from Sichuan, a southwestern region of China. Matching was employed to control for the effect of age and sex for risk of GC (Niven et al., 2012), since they are known potential confounding variables (Crew et al., 2006). We analyzed *IL10*-592 A>C and *IL10*-1082 A>G for GC risk. Because etiologic factors for non-cardia GC may differ from those for cardia subtype (Blaser 1999; McColl et al., 2000; Brown et al., 2002), we disaggregated the patients by subsites (cardia or non-cardia GC) and additionally conducted sub-analysis of the two SNPs for non-cardia GC risk in a 1:1 case-control design.

Materials and Methods

Study population and design

Patients with histologically confirmed GC were enrolled from Yanting Cancer Hospital & Institute in Yanting County, and Sichuan University West China Hospital and Sichuan Cancer Hospital in Chengdu between October 2010 and August 2011. Control subjects were selected from Sichuan University West China Fourth Hospital and a peri-urban community in Chengdu and matched at 1:1 to the cases by age (±3 years) and sex (Wen et al., 2012). The GC cases were categorized according to the location of cancer, and cardia and non-cardia GCs were defined in our earlier study (Wen et al., 2012). We surveyed the subjects using a self-designed questionnaire including demographic factors such as age, sex, and education, and potential risk factors including smoking, alcohol drinking, and family history of cancer. Smokers, drinkers and family history of cancer were defined elsewhere (Wen et al., 2012). Approximately 2-5 ml venous blood was collected from each subject at site. The research was approved by the ethics committees of four participating hospitals, and informed consent was obtained from all recruited subjects.

DNA extraction and SNP genotyping

DNA extraction and SNP genotyping procedures were reported in our earlier study (Wen et al., 2012). Briefly, genomic DNA was extracted 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 assays were designed using Sequenom Assay Design 3.1 software (Table 1). *IL10*-592 A>C and *IL10*-1082 A>G genotypes were analyzed using MALDI-TOF MS according to Justenhoven et al. (Justenhoven et al., 2004) and procedures described in our earlier study (Wen et al., 2012). The MassARRAY Analyzer Compact with ACQUAIRE Module (Sequenom)

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Table 1. Primer Sequences and Masses for Two *IL10* Promoter Polymorphisms

	IL10-592 A>C	*IL10*-1082 A>G
Forward primer	TCCTCAAAGTTCCCAAGCAG	ATTCCATGGAGGCTGGATAG
Reverse primer	AAAGGAGCCTGGAACACATC	GACAACACTACTAAGGCTTC
Un-Extension primer	AGACTGGCTTCCTACAG	CCTATCCCTACTTCCCC
Mass_UEX[a] (Da)	5170.4	4977.2
Variant alleles	A/C	A/G
Mass_EX[b] (Da)	5457.6/5497.5	5224.4/5304.3

[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 (%)	Cases (n=308)	Controls (n=308)	*P* value[a]
Education			
Primary or below	139 (45.4)	144 (46.8)	0.68
Secondary	93 (30.4)	93 (30.2)	
High or technical school	48 (15.7)	55 (17.9)	
College or above	26 (8.50)	16 (5.91)	
Family history of cancer			
No	216 (70.1)	275 (89.3)	<0.01
Yes	92 (29.9)	33 (10.7)	
Smoking			
Non-smokers	128 (41.6)	132 (42.9)	0.74
Smokers	180 (58.4)	176 (57.1)	
Drinking			
Non-drinkers	140 (45.5)	175 (56.8)	<0.01
Drinkers	168 (54.6)	133 (43.2)	

[a]*P* value by the McNemar test

Table 3. Characteristics of Non-cardia GC and Control Subjects

Variables, n (%)	Cases (n=308)	Controls (n=308)	*P* value[a]
Education			
Primary or below	63 (36.2)	79 (44.9)	0.33
Secondary	58 (33.3)	55 (31.3)	
High or technical school	35 (20.1)	30 (17.1)	
College or above	18 (10.3)	12 (6.82)	
Family history of cancer			
No	131 (74.4)	161 (91.5)	<0.01
Yes	45 (25.6)	15 (8.52)	
Smoking			
Non-smokers	77 (43.8)	83 (47.2)	0.52
Smokers	99 (56.3)	93 (52.8)	
Drinking			
Non-drinkers	81 (46.0)	102 (58.0)	0.03
Drinkers	95 (54.0)	74 (42.1)	

[a]*P* value by the Chi-square test

acquired spectra from the SpectroCHIP, which were automatically processed and saved to the MassARRAY database (Wen et al., 2012).

Statistical analysis

Statistical analysis was performed using Stata 8.0 (StataCorp, College Station, USA). We compared demographic characteristics between cases and controls by the McNemar test and Chi-square test. Hardy–Weinberg analysis was performed to compare the observed and expected frequencies of *IL10*-592 A>C and *IL10*-1082 A>G genotypes among the control group using the Chi-square test. Conditional logistic regression was used to calculate odd ratios (OR) and 95% confidence intervals (CIs) for risk of GC. Haplotype analysis was conducted according to previous description (Shi et al., 2005; Li et al., 2009; Wen et al., 2012), and rare haplotypes (< 3%) were excluded from the analysis (Li et al., 2009). All *P* values were two-tailed and statistical significance was indicated by a value of *P*<0.05.

Results

Subject characteristics

We enrolled 308 pairs of GC and control subjects, including 224 (72%) men and 84 (27%) women in each group. The average age was 57.9±10.6 (mean ± SD) years and 57.6±11.1 in the two groups. Demographic information of study subjects have been reported in a previous study (Wen et al., 2012) and summarized in Table 2. There were no statistically significant differences between the GC cases and controls in terms of education

and smoking status, while the proportions of family history of cancer and alcohol drinking were statistically higher among GC patients. We noticed similar results when 176 pairs of non-cardia GC and control subjects were included for sub-analysis (Table 3). We excluded all other GC cases for sub-analysis because only 99 cardia GC cases were identified and the others were diagnosed without information of subsites.

Genotype distributions and their association with overall GC

The genotype distributions of *IL10*-592 A>C and *IL10*-1082 A>G and their association with overall GC are presented in Table 4. Genotyping results demonstrated that the allele frequencies for *IL10*-592C and *IL10*-1082G were 32.5% and 7.95% respectively in cases and 32.0% and 7.63% respectively in controls. The genotype distributions of the two polymorphisms in controls conformed to the Hardy-Weinberg equilibrium (χ^2=0.02, *P*=0.90; χ^2=0.95, *P*=0.33). The allele frequencies of *IL10*-592 A>C and *IL10*-1082 A>G among the cases were not statistically significantly different from those among controls (χ^2=0.03, *P*=0.86; χ^2 =0.05, *P*=0.83). Multivariate regression analyses showed that subjects carrying the *IL10*-592C variant allele had non-significant decreased risk of GC, while those carrying *IL10*-1082G variant allele had non-significant increased risk. Due to the low allele frequencies and relative rarity of the homozygous variant genotypes, we combined the homozygous variant and heterozygous groups for analysis. The distributions of *IL10*-592 A>C and *IL10*-1082 A>G genotypes were not

Table 4. *IL10*-592 A>C and *IL10*-1082 A>G Genotypes and Alleles with Overall GC Risk

SNP	Cases	Controls	OR (95% CI)[a]	
	(n=308)	(n=308)	Crude	Adjusted[b]
IL10-592 A>C				
AA	144 (46.8)	142 (46.1)	1 (reference)	1 (reference)
AC+CC	164 (53.3)	166 (53.9)	0.97 (0.71-1.34)	0.94 (0.66-1.33)
A allele	416 (67.5)	419 (68.0)	1 (reference)	
C allele	200 (32.5)	197 (32.0)	1.02 (0.81-1.30)	
IL10-1082 A>G				
AA	263 (85.4)	264 (85.7)	1 (reference)	1 (reference)
AG+GG	45 (14.6)	44 (14.3)	1.03 (0.66-1.59)	1.00 (0.62-1.60)
A allele	567 (92.1)	569 (92.4)	1 (reference)	
G allele	49 (7.95)	47 (7.63)	0.96(0.63-1.45)	

[a]ORs and 95% CIs were calculated by conditional logistic regression; [b]ORs were adjusted for family history of cancer, drinking, and smoking

Table 5. *IL10*-592 A>C and *IL10*-1082 A>G Genotypes and Alleles with Non-cardia GC Risk

SNP	Cases	Controls	OR (95% CI)[a]	
	(n=176)	(n=176)	Crude	Adjusted[b]
IL10-592 A>C				
AA	94 (53.4)	75 (42.6)	1 (reference)	1 (reference)
AC+CC	82 (46.6)	101 (57.4)	0.63 (0.40-0.98)*	0.58 (0.36-0.95)*
A allele	250 (71.0)	235 (66.8)	1 (reference)	
C allele	102 (29.0)	117 (33.2)	0.82 (0.60-1.13)	
IL10-1082 A>G				
AA	154 (87.5)	148 (84.1)	1 (reference)	1 (reference)
AG+GG	22 (12.5)	28 (15.9)	0.76 (0.42-1.38)	0.73 (0.38-1.40)
A allele	329 (93.5)	322 (91.5)	1 (reference)	
G allele	23 (6.53)	30 (8.52)	0.75 (0.43-1.32)	

[a]ORs and 95% CIs were calculated by conditional logistic regression; [b]ORs were adjusted for family history of cancer, drinking, and smoking; *P<0.05

statistically significant between the cases and controls (χ^2 =0.03, P=0.88; χ^2 =0.01, P=0.91). The variant genotypes of *IL10*-592 A>C (adjusted OR, 0.94; 95% CI, 0.66-1.33) and *IL10*-1082 A>G (adjusted OR, 1.00; 95% CI, 0.45-1.61) were not associated with GC risk.

Subanalysis of genotype distributions and their association with non-cardia GC

The genotype distributions of *IL10*-592 A>C and *IL10*-1082 A>G and their association with non-cardia GC are presented in Table 5. Genotyping results demonstrated that the allele frequencies for *IL10*-592C and *IL10*-1082G, respectively, were 29.0% and 6.53% in non-cardia cases and 33.2% and 8.52% in controls. The genotype distributions of two polymorphisms in the matched control conformed to the Hardy-Weinberg equilibrium (χ^2=1.37, P=0.24; χ^2=0.49, P=0.49). The allele frequencies of *IL10*-592 A>C were not statistically significantly different from those in controls (χ^2=1.49, P=0.22; χ^2=1.00, P =0.32). Multivariate regression analyses showed that subjects carrying the *IL10*-1082G variant allele had a non-significant decreased risk. The distributions of *IL10*-592 A>C were statistically significant between the cases and controls (χ^2 =4.11, P=0.04). The variant genotype of *IL10*-592 A>C was associated with decreased non-cardia GC risk (adjusted OR, 0.58; 95% CI, 0.36-0.95).

We analyzed the interaction between *IL10*-592 A>C genotype and smoking or drinking for risk of non-cardia GC. No interaction was observed between the *IL10*-592

Table 6. Distribution of *IL10* Haplotypes and Non-cardia GC

Haplotypes*	Case (%)	Control (%)	χ^2	P value	OR(95%CI)
A-G	0.00 (0.00)	0 (0.00)	-	-	-
A-A	250 (71)	235 (66.80)	1.49	0.22	1.22 (0.89-1.68)
C-A	79 (22.4)	87 (24.7)	0.51	0.45	0.88 (0.62-1.25)
C-G	23 (6.50)	30 (8.50)	1	0.32	0.75 (0.43-1.32)
Global frequency	-	-	1.77	0.41	-

*In the order of rs1800872 (*IL10*-592 A>C) and rs1800896 (*IL10*-1082A>G). Rare haplotypes (< 3%) were excluded from the analysis

genotype and smoking (adjusted OR, 1.60; 95% CI, 0.60-4.23) or drinking (adjusted OR, 1.58; 95% CI, 0.64-3.92), which agrees with our finding in an earlier SNP analysis (Wen et al., 2012).

Association of IL10 haplotypes and non-cardia GC risk

To evaluate the extent of LD, the statistics of D' and r^2 between all possible pairs of *IL10*-592 A>C and *IL10*-1082 A>G polymorphisms were calculated. It was observed that *IL10*-592 A>C and *IL10*-1082 A>G were in strong LD (D'=1.00, r^2=0.18). Haplotype analysis was performed on haplotypes constructed of two SNPs using the SHEsis software. Three major haplotypes (> 3% in the control group) were identified in both non-cardia GC cases and controls. The distributions of the different haplotypes in each group are shown in Table 6. No association was found between any of the *IL10* haplotypes and risk of non-cardia GC.

Discussion

IL-10 generally functions as an immunosuppressor and anti-inflammatory mediator, and has been implicated in autoimmune diseases and the progression of malignancies (Mocellin et al., 2005; Yuzhalin 2011). Since Wu et al. (2003) and El-Omar et al. (2003) concurrently reported *IL10* promoter polymorphisms were associated with GC risk, a number of case-control studies have been conducted to evaluate the association between *IL10* promoter polymorphisms and GC in humans. In this matched case-control study, we analyzed genetic polymorphisms of *IL10* for GC risk in Han Chinese in a southwestern region of China. The main finding in our study was that *IL10*-592 AC/CC genotypes were significantly associated with a decreased risk of developing non-cardia GC, while the *IL10*-1082 polymorphism had no association. The *IL10*-592 and *IL10*-1082 polymorphisms were in strong LD, but the haplotypes established by these two polymorphisms were not associated with non-cardia GC risk.

Both *IL10* polymorphisms were not associated with overall GC risk in our study. Our finding is consistent with those of several other studies (El-Omar et al., 2003; Guo et al., 2005; Zambon et al., 2005; Bai et al., 2008; Con et al., 2009; Shin et al., 2011). Of a few studies that have reported otherwise, only three have reported associations of statistical significance with GC for *IL10*-592 AC/CC (Wu et al., 2003) and *IL10*-1082 AG/GG (Wu et al., 2003; Lu et al., 2005; Zhou et al., 2011). Since these studies were conducted in different populations, direct comparisons between them are difficult to make. It can be

postulated that the discrepancies may be due to differences in variant frequencies between races, and that *IL10* gene polymorphisms can differentially affect GC development between populations (Won et al., 2010). Of note, we found that all three studies with contrasting significant results were undertaken in China (Wu et al., 2003; Lu et al., 2005; Zhou et al., 2011), and that their results were consistent with those in the Chinese from a meta-analysis (Loh et al., 2009). It is reasonable to hypothesize that intra-racial differences may have a role in our observed results, given that China is such a large country with varied environmental exposures and dietary habits. The source populations in these studies on Chinese were mainly from Chinese Taiwan (Wu et al., 2003) and Northern China (Lu et al., 2005), which may be different from our current study population in Southwestern China. This variability in GC risk associated with genetic polymorphisms within different geographic areas of China is corroborated in our previous study (Loh et al., 2009). One study from a population in the same western region of China found that *IL10*-1082 AG/GG was associated with an increased malignancy risk (Lu et al., 2005). However, the frequency of the variant genotype was 10.7% in their control group, which was lower than the 14.3% observed in our study. The source of the control population was not indicated in the former study, and it is possible that the controls were selected from the patients in the hospital, which differs from our community-based design.

In sub-analysis, we found that the *IL10*-592 AC/CC polymorphism was associated with reduced risk of non-cardia GC, in contrast to the lack of association observed in the overall cohort. This may reflect known differences in the etiology, pathology, carcinogenesis, and prognosis of cardia and non-cardia GC (Ni et al., 2012), and highlights the potential of masking or underestimating associations in analyzing a GC cohort combining the two subtypes. The inconsistency between non-cardia and overall GC results could be attributable to variations in association with respective GC subtypes. This possibility is also indirectly supported by the finding that patients with cardia GC had a significantly lower frequency of *IL10*-592 AA genotype than those with non-cardia GC (Zhuang et al., 2010). In addition, our finding of a lack of association of *IL10*-1082 AG/GG with non-cardia malignancy agrees with a meta-analysis on the non-cardia subtype (Ni et al., 2012). Nevertheless, caution should be taken when interpreting the significance of these findings, since non-cardia GC cases and controls in this study may potentially not be representative of the source population due to the post-hoc nature of the analysis. A large well-designed case-control study with a priori hypotheses regarding non-cardia GC risk may allow the results to be more generalizable.

It is plausible that an *IL10*-592 AC/CC genotype could determine a reduced risk of non-cardia GC. *IL10* polymorphisms have been shown to influence the transcription of *IL10* messenger RNA and the expression of IL-10 in vitro (Turner et al., 1997; Gibson et al., 2001), and have been reported to determine inter-individual differences in the IL-10 production (Liu et al., 2011). They have also been found to correlate with expression of mucosal IL-10 in patients with HP infections (Rad et

al., 2004). The GCC haplotype for *IL10*-1082/-819/-592 polymorphisms has been associated with higher IL-10 production than the ATA haplotype (Yuzhalin 2011), suggesting an enhanced anti-inflammatory capacity for *IL10* -592 C allele carriers. The anti-inflammatory capacity may help to moderate the recognized tumorigenic effects of inflammation, and hence determine a reduced risk of GC for *IL10* -592 C allele carriers. Moreover, these effects may be enhanced in Asians in which the HP infection rate is high and premalignant stages of intestinal metaplasia and dysplasia are common (Seno et al., 2007), compared to Caucasians who generally have a low prevalence of premalignant lesions and a low HP infection rate (Won et al., 2010). The status of HP infection in the association sub-analysis would have been thus important to consider, but unfortunately we were not able to do so in this study. This may signal a future direction for analysis of *IL10* SNPs for risk of overall GC or GC of subsites.

It was observed that *IL10*-592 A>C and *IL10*-1082 A>G were in strong LD, which is consistent with several other studies (Turner et al., 1997; Crawley et al., 1999; Alpizar-Alpizar et al., 2005; Liu et al., 2011). However, no risk of non-cardia GC was found for established *IL10* haplotypes. Since a polymorphism may be associated with GC risk if it is in linkage disequilibrium with a causative polymorphism in the same or a tightly linked gene (Sicinschi et al., 2006), it may be important to explore the linkage disequilibrium of *IL10*-592 polymorphism with other funtionionally related genes to account for the real causative polymorhism.

In conclusion, our data do not link the two SNPs of *IL10*-592 and *IL10*-1082 with overall GC risk. We demonstrate that *IL10*-592 polymorphism is associated with protective effect against non-cardia GC in our study population. Haplotype analysis did not identify any haplotype associated with GC risk. Our results should be confirmed in future large population-based studies or prospective studies before they are extropolated. Since IL-10 can both reduce and enhance anti-cancer properties, it may be of significance to explore the role of *IL10* polymorphisms in the development of GC in different clinical stages, or GC of different subsites.

Acknowledgements

References

Akdis M, Burgler S, Crameri R, et al (2011). Interleukins, from 1 to 37, and interferon-gamma: receptors, functions, and roles in diseases. *J Allergy Clin Immunol*, **127**, 701-21 e1-70.

Alpizar-Alpizar W, Perez-Perez GI, Une C, et al (2005). Association of interleukin-1B and interleukin-1RN

polymorphisms with gastric cancer in a high-risk population of Costa Rica. *Clin Exp Med*, **5**, 169-76.

Bai XL, Sun LP, Liu J, et al (2008). Correlation of interleukin-10-1082G/a single nucleotide polymorphism to the risk of gastric cancer in north China: a case-control study [in Chinese]. *Chin J Cancer*, **27**, 35-40.

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.

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

Con SA, Takeuchi H, Con-Chin GR, et al (2009). Role of bacterial and genetic factors in gastric cancer in Costa Rica. *World J Gastroenterol*, **15**, 211-8.

Crawley E, Kay R, Sillibourne J, et al (1999). Polymorphic haplotypes of the interleukin-10 5' flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. *Arthritis Rheum*, **42**, 1101-8.

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

El-Omar EM, Rabkin CS, Gammon MD, et al (2003). Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology*, **124**, 1193-201.

Ferlay J, Shin HR, Bray F, et al GLOBOCAN 2008 v2.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10 [Internet], Lyon, France: International Agency for Research on Cancer; 2010. Available from: http://globocan.iarc.fr, accessed on 25/2/2013.

Gibson AW, Edberg JC, Wu J, et al (2001). Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. *J Immunol*, **166**, 3915-22.

Guo W, Wang N, Wang YM, et al (2005). Interleukin-10 -1082 promoter polymorphism is not associated with susceptibility to esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma in a population of high-incidence region of north China. *World J Gastroenterol*, **11**, 858-62.

Jemal A, Center MM, Desantis C, Ward EM (2010). Global patterns of cancer incidence and mortality rates and trends. *Cancer Epidemiol Biomarkers Prev*, **19**, 1893-907.

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, 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.

Li Z, Zhang Z, He Z, et al (2009). A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). *Cell Res*, **19**, 519-23.

Liu J, Song B, Wang JL, et al (2011). Polymorphisms of interleukin-10 promoter are not associated with prognosis of advanced gastric cancer. *World J Gastroenterol*, **17**, 1362-7.

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.

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

Mocellin S, Marincola FM, Young HA (2005). Interleukin-10 and the immune response against cancer: a counterpoint. *J Leukoc Biol*, **78**, 1043-51.

Ni P, Xu H, Xue H, et al (2012). A meta-analysis of interleukin-10-1082 promoter polymorphism associated with gastric cancer risk. *DNA Cell Biol*, **31**, 582-91.

Niven DJ, Berthiaume LR, Fick GH, Laupland KB (2012). Matched case-control studies: a review of reported statistical methodology. *Clin Epidemiol*, **4**, 99-110.

Rad R, Dossumbekova A, Neu B, et al (2004). Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during Helicobacter pylori infection. *Gut*, **53**, 1082-9.

Salazar-Onfray F, Lopez MN, Mendoza-Naranjo A (2007). Paradoxical effects of cytokines in tumor immune surveillance and tumor immune escape. *Cytokine Growth Factor Rev*, **18**, 171-82.

Seno H, Satoh K, Tsuji S, et al (2007). Novel interleukin-4 and interleukin-1 receptor antagonist gene variations associated with non-cardia gastric cancer in Japan: Comprehensive analysis of 207 polymorphisms of 11 cytokine genes. *J Gastroenterol Hepatol*, **22**, 729-37.

Shi YY, He L (2005). SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res*, **15**, 97-8.

Shin CM, Kim N, Lee HS, et al (2011). Intrafamilial aggregation of gastric cancer: a comprehensive approach including environmental factors, Helicobacter pylori virulence, and genetic susceptibility. *Eur J Gastroenterol Hepatol*, **23**, 411-7.

Sicinschi LA, Lopez-Carrillo L, Camargo MC, et al (2006). Gastric cancer risk in a Mexican population: role of Helicobacter pylori CagA positive infection and polymorphisms in interleukin-1 and -10 genes. *Int J Cancer*, **118**, 649-57.

Turner DM, Williams DM, Sankaran D, et al (1997). An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet*, **24**, 1-8.

Wang J, Ding Q, Shi Y, et al (2012). The interleukin-10-1082 promoter polymorphism and cancer risk: a meta-analysis. *Mutagenesis*, **27**, 305-12.

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.

Won HH, Kim JW, Kim MJ, et al (2010). Interleukin 10 polymorphisms differentially influence the risk of gastric cancer in East Asians and Caucasians. *Cytokine*, **51**, 73-7.

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.

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

Zambon CF, Basso D, Navaglia F, et al (2005). Pro- and anti-inflammatory cytokines gene polymorphisms and Helicobacter pylori infection: interactions influence outcome. *Cytokine*, **29**, 141-52.

Zhou Y, Hu W, Zhuang W, Wu X (2011). Interleukin-10 -1082 promoter polymorphism and gastric cancer risk in a Chinese Han population. *Mol Cell Biochem*, **347**, 89-93.

Zhuang W, Wu XT, Zhou Y, et al (2010). Interleukin10 -592 promoter polymorphism associated with gastric cancer among Asians: a meta-analysis of epidemiologic studies. *Dig Dis Sci*, **55**, 1525-32.