GASTRIC CANCER

Association of *interleukin 1B* gene polymorphism and gastric cancers in high and low prevalence regions in China

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Aim: Our aim was to study the relationship between interleukin 1B (*IL-1B*) polymorphism, *Helicobacter pylori* infection, and gastric cancer in high prevalent (Shanxi) and low prevalent (Guangdong) regions in China.

Method: Genomic DNA was extracted from peripheral blood of 192 healthy volunteers, 84 gastric cancer patients from Guangdong and 169 healthy volunteers, and 86 gastric cancer patients from Shanxi. Polymorphisms in *IL-1B* that encodes IL-1 β and *IL-1RN* that encodes IL-1 receptor antagonist were analysed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). These polymorphic sites include promoter regions of *IL-1B* at positions +3954, -511 (C-T transition), and -31 (T-C transition), and *IL-1RN* variable tandem repeats.

Results: In the low prevalence region, the frequencies of the *IL-1B*+3954 T/T and *IL-1RN**2/*2 genotypes were similar. *IL-1B* -511T/T genotype frequency was significantly higher among patients with gastric cancer (25.0%) than control subjects (12.5%) ($\chi^2=6.7$, $p=0.01$). In the high prevalence region, the frequencies of the *IL-1B*+3954T/T and -511T/T genotypes and the *IL-1RN**2/*2 genotype in the cancer and control groups were similar. *IL-1B* -31C/C genotype frequency was significantly higher among patients with gastric cancer (90.0%) than controls (78.0%) ($\chi^2=5.0$, $p=0.025$). Compared with the low prevalence region, control subjects from the high prevalence region had a higher frequency of the *IL-1B* -511T/T genotype (23.0% v 12.5%; $\chi^2=7.0$, $p<0.008$). While *H. pylori* infection alone had only a modest effect on the risk of gastric cancer development (odds ratio (OR) 5.0 (95% confidence interval (CI) 1.5-16.3)), combined with the *IL-1B* -511T/T genotype the risk was markedly elevated (OR 17.1, 95% CI 3.8-76.4).

Conclusion: *IL-1B* -511T/T genotypes are associated with gastric cancer in China. The effect of *IL-1B* polymorphism is less obvious in areas of high prevalence for gastric cancer.

Helicobacter pylori infection causes a wide spectrum of gastric pathologies, from asymptomatic gastritis and peptic ulcer disease to gastric malignancies. The rationale for this divergent clinical outcome is gradually unveiled in the paradigm of the two way interaction between acid secretion and *H. pylori* induced gastritis.¹ The acid secreting capacity of the stomach is crucial in determining outcome: a high acid secretor with antral predominant gastritis tends to develop peptic ulcers whereas a low secretor with pangastritis tends to develop gastric cancer.

Recently, the interleukin (*IL*)-1*B* gene has been proposed as a key factor in determining the pattern of gastritis and risk of malignant transformation.² IL-1 β , induced by *H. pylori* infection, is known to be a strong proinflammatory cytokine as well as a strong inhibitor of acid secretion in the stomach. IL-1 gene cluster (*IL-1B* encoding IL-1 β and *IL-1RN* encoding the IL-1 receptor antagonist) has a number of functionally relevant polymorphisms that could be correlated with high or low IL-1 β production.³ El-Omar *et al* first reported that genotypes *IL-1B*-511T+/-31C+ and *IL-1RN**2/*2 are associated with an increased risk of gastric cancer development.⁴ Their findings in Scottish and Polish patients were subsequently confirmed by studies in other ethnic groups from the USA⁵ and Portugal.⁶ In Machado *et al*'s study, the association between proinflammatory IL-1 genotypes and gastric cancer was confined to the intestinal type with only a non-significant risk being shown for the diffuse type.⁶ Furthermore, in a Japanese study, proinflammatory *IL-1B* polymorphisms were also found to be associated with hypochlorhydria and atrophic gastritis, a precancerous lesion in the stomach.⁷ These abnormalities were correlated with

reduction in serum pepsinogen I/II ratio which has been established as a surrogate marker of gastric atrophy. The host genetic makeup and bacterial genotypes were investigated in first degree relatives of the Scottish gastric cancer cohort. Atherton *et al* reported that putative high output *IL-1B* genotypes and *vacA* s1/m1*H. pylori* type are independently associated with hypochlorhydria and gastric atrophy.⁸

It is known that gastric cancer is prevalent in the northern part of China whereas peptic ulcer disease is more common in the south.⁹ Factors such as the prevalence of *H. pylori* infection and environmental factors, including diet and smoking, have been implicated in the different disease patterns. In the present study, we investigated the genotype of gastric cancer patients and healthy volunteers in two regions of China with different prevalence rates of gastric cancer.

METHOD

Subjects

Two provinces were chosen in this study to represent areas of high prevalence (Shanxi) and low prevalence (Guangdong) for gastric cancer in China. Their mean annual gastric cancer mortality is approximately 23.6 and 7.9/100 000 population.⁹⁻¹⁰ In Guangdong, a total of 276 subjects were studied. This included 84 patients with histologically confirmed non-cardiac gastric cancer and 192 healthy volunteers from the Sun Yat-sen University of Medical Sciences. In Shanxi

Abbreviations: IL, interleukin; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; OR, odds ratio

Table 1 Demographic data and *Helicobacter pylori* status of subjects in Guangdong (low prevalence region) and Shanxi (high prevalence region)

	Guangdong (Low prevalence region)		Shanxi (High prevalence region)	
	Controls	GC patients	Controls	GC patients
No of subjects	192	84	169	86
Age (y) (mean (SD))	21.2 (1.35)	56.1 (14.2)	21.8 (1.95)	60.8 (10.2)
Female (%)	119 (61.9%)	41 (48.8%)	108 (63.9%)	31 (36.0%)
<i>H. pylori</i> positive (%)	67 (34.9%)	68 (81.0%)	83 (49.1%)	74 (86.1%)
(sex adjusted rate)*	(36.0%)	(81.0%)	(49.1%)	(85.0%)

**H. pylori* infection rate was adjusted according to the fifth population census of China.
GC, gastric cancer.

province, 86 patients with non-cardiac gastric cancer and 169 healthy volunteers from Xian Medical University were recruited into the study. Age and sex of the patients are shown in table 1. All subjects included in this study belonged to the ethnic group of Han. None of these subjects had a history of systemic lupus erythematosus, diabetes mellitus, rheumatoid arthritis, or inflammatory bowel disease. Subjects with a family history of gastric cancer were also excluded.

Evaluation of *H. pylori* prevalence

H. pylori status of subjects was determined by an ELISA assay for anti-*H. pylori* IgG (BioChek Inc., USA). All analyses were done in duplicate and with an internal standard. Concentrations of *H. pylori* IgG were determined by reading at an optical density of 450 nm. Subjects were considered to be *H. pylori* positive if the concentration exceeded 20 U/ml.

DNA extraction

DNA was isolated from peripheral blood using the NaI method. Briefly, heparinised whole blood (100 µl) was added to equal volumes of 6 M NaI and chloroform:isoamle alcohol, and centrifuged at 5000 *g* for five minutes. The aqueous layer was removed and isopropanol was added to the pellet to deposit DNA (centrifuged at 5000 *g* for five minutes). Extracted DNA was rinsed 2~3 times with 70% alcohol and resuspended in 40 µl of TE buffer (pH 8.0).¹¹

Genotyping of IL-1 polymorphisms

Polymorphisms in *IL-1B* that encodes IL-1β and *IL-1RN* that encodes IL-1 receptor antagonist were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *IL-1B* polymorphic sites include the promoter region of *IL-1B* at positions +3954, -511 (C-T transition), and -31 (T-C transition) (fig 1).

A fragment containing the *Ava*I polymorphic site at position -511 of the *IL-1B* gene was amplified by PCR. The oligonucleotides 5'-GCCTGAACCCTGCATACCGT-3' and 5'-GCCAATAGCCCTCCCTTCT-3' flanking this region were used as primers. PCR conditions were as follows: denaturation at 94°C for 10 minutes, followed by 94°C (30 seconds), 65°C (30 seconds), and 72°C (30 seconds) for five cycles, 94°C (30 seconds), 60°C (30 seconds), and 72°C (30 seconds) for 30 cycles, followed by 94°C (30 seconds), 55°C (30 seconds), and 72°C (30 seconds) for five cycles. A final extension was carried out at 72°C for seven minutes and cooling down to 4°C. Fragments were separated by electrophoresis on 3% agarose with ethidium bromide staining using appropriate commercially available size markers for comparison. The C allele was designated if two bands of 92 and 63 bp were obtained, and the T allele was designated if a signal band of the undigested 155 bp was obtained. Genotypes were designated as follows: C/C, two bands of 92 and 63 bp; C/T, three bands of 155, 92, and 63 bp; and T/T, a single band of 155 bp.

A fragment containing the *Alu*I polymorphic site at position -31 of the *IL-1B* gene was amplified by PCR. The oligonucleotides 5'-AGAAGCTTCCACCAATACTC-3' and 5'-ACCACCTAGTTGTAAGGAAG-3' flanking this region were used as primers. PCR conditions were the same as above. Electrophoresis on 3% agarose with ethidium bromide staining was used. The T allele was designated if two bands of 100 and 52 bp were obtained and the C allele was designated if a single band of the undigested 152 bp was obtained. Genotype were designated as follows: T/T, two bands of 100 and 52 bp; C/T, three bands of 152, 100, and 52 bp; and T/T, a single band of 100 bp.

A fragment containing the *Taq*I polymorphic sites at position +3954 of the *IL-1B* gene was amplified by PCR. The oligonucleotides 5'-AATTTGGCACCTCGCCTCA-3' and 5'-CGGAGCGTGCAGTTCAGTGAT-3' flanking this region were used as primers. PCR conditions were the same as above. Electrophoresis on 3% agarose with ethidium bromide staining was used. The C allele was designated if two bands of 88 and 64 bp were obtained and the T allele was designated if a single band of undigested 152 bp was obtained. Genotypes were designated as follows: C/C, two

Figure 1 Genotyping of the *IL-1B* 511C/T and *IL-1RN* variable number of tandem repeats polymorphism. (A) Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) agarose gel electrophoresis of the *IL-1B* 511C/T polymorphism showing the C/T, C/C, and T/T genotypes. (B) PCR-RFLP agarose gel electrophoresis of the *IL-1RN* variable number of tandem repeats polymorphism illustrating the most commonly studied genotypes.

Table 2 *IL-1B* genotype frequencies in control subjects and gastric cancer (GC) patients in Guangdong (low prevalence region) and Shanxi (high prevalence region)

		Guangdong (low prevalence region)		Shanxi (high prevalence region)	
Genotype		Controls (n = 192)	GC patients (n = 84)	Controls (n = 169)	GC patients (n = 86)
<i>IL-1B</i> -511	C/C	87	18	34	19
	C/T	78	45	97	45
	T/T	27 (14.1%)	21 (25.0%)	38 (22.5%)	22 (25.6%)
<i>IL-1B</i> -31	Sex adjusted T/T	(12.5%)	(25.0%)*	(23.0%)‡	(23.1%)
	C/C	156 (81.3%)	54 (64.3%)	128 (75.7%)	78 (90.7%)
	Sex adjusted C/C	(82.1%)	(64.3%)†	(78.0%)	(90.0%)§
<i>IL-B</i> +3954	C/T	35	27	34	4
	T/T	1	3	7	4
	C/C	164	77	152	77
	C/T	28	7	17	8
	T/T	0	0	0	1 (1.2%)

*Cancer_{Guangdong} versus control_{Guangdong}: $\chi^2 = 6.7$, $p = 0.010$, OR 2.3, 95% CI 1.2–4.5.†Cancer_{Guangdong} versus control_{Guangdong}: $\chi^2 = 9.9$, $p = 0.002$, OR 2.5, 95% CI 1.4–4.4.‡Control_{Guangdong} versus control_{Shanxi}: $\chi^2 = 7.0$, $p = 0.008$, OR 2.1, 95% CI 1.2–3.7.§Cancer_{Shanxi} versus control_{Shanxi}: $\chi^2 = 5.0$, $p = 0.025$, OR 2.4, 95% CI 1.1–5.2.**Table 3** *IL-1RN* genotype frequencies in control subjects and gastric cancer (GC) patients in Guangdong (low prevalence region) and Shanxi (high prevalence region)

		Guangdong (low prevalence region)		Shanxi (high prevalence region)	
Genotype		Controls (n = 192)	GC patients (n = 84)	Controls (n = 169)	GC patients (n = 86)
<i>IL-1RN</i>	*1/*1	175	74	161	76
	*1/*2	13	8	4	9
	*2/*2	2	0	2	1
	*1/*3	2	0	1	0
	*1/*4	0	1	1	0
	*2/*4	0	1	0	0
<i>IL-1RN</i>	L/L	177	75	163	76
	L/*2	13	9	4	9
	*2/*2	2 (1.0%)	0	2 (1.2%)	1 (1.2%)
	Sex adjusted *2/*2	(1.1%)		(0.9%)	(1.6%)

bands of 88 and 64 bp; C/T, three bands of 152, 88, and 64 bp; and T/T, a single band of 152 bp.

The *IL-RN* gene has a penta-allelic 86 base pair tandem repeat polymorphism (variable number of tandem repeats). The less common allele 2 (*IL-1RN**2) is associated with enhanced *IL-1β* production and a wide range of chronic inflammatory and autoimmune conditions. The *IL-RN* polymorphism was analysed by PCR that amplifies the tandem repeat regions, as described previously.⁴ Oligonucleotides 5'-CCCCTCAGCAACTCC-3' and 5'-GGTCAGAAGGGCAGAGA-3' were used as primers in the PCR. The PCR conditions were the same as above. PCR products were sized by electrophoresis on a 2% agarose gel stained with ethidium bormide. Alleles were sized relative to a 100 bp DNA ladder and coded as follows: allele 1 = 410 bp (four repeats), allele 2 = 240 bp (two repeats), allele 3 = 530 bp (five repeats), allele 4 = 325 bp (three repeats), and allele 5 = 595 bp (six repeats). These alleles were then classified as short (allele 2* = *2) or long (alleles *1, *3, *4 and *5 = L). The genotyping patterns were classified as L/L, L/*2, and *2/*2 in accordance with the recent report by Machado and colleagues.⁶

Statistical analysis

The sex difference in the two populations was adjusted according to the fifth population census of China.¹² The Hardy-Weinberg equilibrium at individual loci was assessed by χ^2 statistics using the program SPSS (version 10.1, Chicago, Illinois, USA). Comparison of genotype frequencies

between cases and controls was assessed by χ^2 statistics. The effect of the *IL-1B*-511T allele on the risk of gastric cancer was expressed as odds ratios (OR) with 95% confidence interval (CI). All p values were two sided, and only values <0.05 were considered statistically significant.

RESULTS

Mean age and sex of the gastric cancer patients and control subjects are shown in table 1. As the control subjects were medical students from Sun Yat-sen University of Medical Sciences and Xian Medical University, their ages were much lower than those of the gastric cancer patients. There was also a predominance of females in the control groups.

The frequencies of genotypes *IL-1B* and *IL-1RN* are summarised in tables 2 and 3, respectively. In the low

Table 4 Allele frequency of *IL-1B*-511 and *IL-1B*-31 in low prevalence and high prevalence regions for gastric cancer

Allele linkage (<i>IL-1B</i> -511/-31)	Non-cancer subjects in low prevalence region (n = 192)	Non-cancer subjects in high prevalence region (n = 169)
C-C	0.61	0.44
C-T	0.05	0.04
T-C	0.32	0.46
T-T	0.02	0.06

prevalence region for gastric cancer (Guangdong), there was no difference in the frequencies of the *IL-1B*+3954 T/T and *IL-1RN* *2/*2 genotypes between gastric cancer patients and controls. The *IL-1RN* *2/*2 genotype was not found in any gastric cancer patient in this region. The *IL-1B*-511 T/T genotype was significantly more frequent in gastric cancer patients (25.0%) compared with controls (12.5%) ($\chi^2 = 6.7$, $p = 0.01$). The risk of developing gastric cancer with this genotype was significantly increased (OR 2.3, 95% CI 1.2–4.5). In this cohort, the prevalence of the *IL-1B* -31C/C genotype in control subjects was significantly higher than that in gastric cancer patients (82.1% v 64.3%; $\chi^2 = 9.9$, $p = 0.002$).

Unlike the findings in the low prevalence region, the high prevalence region showed no difference in the distribution of *IL-1B* +3954 T/T, -511 T/T, or *IL-1RN* genotype between gastric cancer patients and controls. The *IL-1RN* *2/*2 genotype was found in only one gastric cancer patient (1.2%) and two control subjects (1.2%). The *IL-1B*-511 T/T genotype was found in 23.1% of gastric cancer patients and 23.0% of control subjects. There was no difference in the frequency of this proinflammatory genotype between the two groups (23.1% v 23.0%; $\chi^2 = 0.02$, $p = 0.89$). Comparing control subjects in high prevalence versus low prevalence regions for gastric cancer, the frequency of the *IL-1B* -511 T/T genotype was significantly higher in the former (23.0% v 12.5%; $\chi^2 = 7.0$, $p = 0.008$). However, the prevalence of the *IL-1B* -31C/C genotype in gastric cancer patients in the high prevalence region was significantly higher than that of control subjects (90.0% v 78.0%; $\chi^2 = 5.0$, $p = 0.025$).

As *IL-1B*-511 and *IL-1B*-31 show differences in frequencies between cancer patients and control subjects and between high prevalence and low prevalence regions, we tested linkage disequilibrium for the two polymorphisms in the two study regions. The results are summarised in table 4 and show that for the *IL-1B* -511 and -31 genotypes there was no evidence of linkage disequilibrium in both the high prevalence and low prevalence regions ($D' = 0.08$ and 0.11 ; $\chi^2 = 2.12$ and 4.14 , $p > 0.05$). The two genotypes were independent risk factors for gastric carcinogenesis in both regions.

The prevalence of *H pylori* infection in the two regions is given in table 1. In the low prevalence region of Guangdong, patients with gastric cancer had significantly higher rates of infection compared with controls (81.0% v 36.0%; $\chi^2 = 49.6$, $p < 0.001$, OR 7.93, 95% CI 4.27–14.74). The same was true for the high prevalence region of Shanxi where *H pylori* infection was more common among patients with gastric cancer than controls (85.0% v 49.1%; $\chi^2 = 32.9$, $p < 0.001$, OR 6.39, 95% CI 3.24–12.62). As genotype *IL-1B* -511 T/T appears to have the most consistent association with gastric cancer, analysis of the cancer risk in relation to *H pylori* infection and this genotype was performed (tables 5, 6). In the low prevalence area for gastric cancer, using non-infected (*H pylori*-) cases

and T/T genotype as the reference group, the risk of gastric cancer in the non-infected and T/T genotype was not significantly raised ($\chi^2 = 0.7$, $p = 0.4$). However, in the presence of *H pylori* infection, the C/C genotype had an increased risk of developing gastric cancer ($\chi^2 = 7.9$, $p = 0.005$). The risk of cancer development was dramatically increased for the T/T genotype with *H pylori* infection ($\chi^2 = 16.9$, $p < 0.001$, OR 17.1, 95% CI 3.8–76.4). In the high prevalence region, the non-infected T/T genotype did not have a significantly increased risk of gastric cancer ($\chi^2 = 1.1$, $p = 0.29$). *H pylori* infection in the C/C genotype had a significantly raised risk of gastric cancer ($\chi^2 = 13.3$, $p < 0.001$, OR 22.8, 95% CI 2.7–190.8). *H pylori* infection in the T/T genotype also had a significantly increased risk of gastric cancer ($\chi^2 = 6.1$, $p = 0.014$, OR 5.0, 95% CI 1.3–20.3).

DISCUSSION

Gastric cancer, like many malignancies, is a result of interaction between genetic factors of the host together with dietary and other factors in the environment. Epidemiological studies on Northern Chinese and American Japanese in Hawaii lent strong support to the effects of lack of fresh fruit and vegetable, smoking, and consumption of salty food in the development of gastric cancer.^{13–14} In the last decade, much attention has been focused on the role of *H pylori* infection in the development of the disease. Correa, in his multistep multifactorial model of gastric carcinogenesis, has proposed that *H pylori* plays a pivotal role in triggering chronic inflammation of the stomach leading to stepwise development of the malignancy.¹⁵ Recently, a host genetic factor, *IL-1B* polymorphism, has been added as the last piece in the jigsaw of gastric carcinogenesis. The biological effects of *IL-1B* as a potent proinflammatory cytokine and inhibitor of acid suppression fits with Correa's model. In the landmark study by El-Omar *et al*, the estimated odds ratios for individuals having the *IL-1B*-511T+/-31C+ and *IL-1RN**2/*2 genotypes of developing gastric cancer were 1.6 and 2.9, respectively.⁴ This finding was subsequently confirmed in other ethnic groups in the USA and Europe.^{5–6}

In contrast with studies in Western countries, the relationship between *IL-1B* polymorphism and gastric cancer has been less clearcut in studies from Asia. In Korea, Ryu *et al* reported that *IL-1B* polymorphisms at loci -511 and -31 were not associated with *H pylori* infection and the risk of gastric cancer.¹⁶ Furuta *et al* reported that the *IL-1B*-511 genotype was associated with high gastric juice pH in *H pylori* infected subjects but the effects of the *IL-1RN* polymorphism were insignificant.⁷ They also reported that the allele 2 gene was extremely uncommon in Japanese. Kato *et al* failed to demonstrate a positive association between the *IL-1B* -511C allele and gastric cancer in their Japanese cohort of 699 subjects.¹⁷

In our study, we chose Shanxi and Guangdong to represent areas of high prevalence and low prevalence of gastric cancer

Table 5 Odds ratio (OR) of developing gastric cancer according to *IL-1B* -511 genotypes and *Helicobacter pylori* status in Guangdong (low prevalence region)

<i>H pylori</i> status	<i>IL-1B</i> -511	Control (n=192)	Cancer (n=84)	χ^2	p Value	OR (95% CI)
<i>H pylori</i> -	C/C	51	4			
	T/T	20	3	0.7	0.4*	NS
<i>H pylori</i> +	C/C	36	14	7.9	0.005†	5.0 (1.5–16.3)
	T/T	7	18	16.9	<0.001‡	17.1 (3.8–76.4)

*Cancer_{*Hp*-} T/T versus control_{*Hp*-} T/T: $\chi^2 = 0.7$, $p = 0.4$.

†Cancer_{*Hp*+} C/C versus control_{*Hp*+} C/C: $\chi^2 = 7.9$, $p = 0.005$, OR 5.0, 95% CI 1.5–16.3.

‡Cancer_{*Hp*+} T/T versus Control_{*Hp*+} T/T: $\chi^2 = 16.9$, $p < 0.001$, OR 17.1, 95% CI 3.8–76.4

Table 6 Odds ratio (OR) of developing gastric cancer according to *IL-1B* –511 genotypes and *Helicobacter pylori* status in Shanxi (high prevalence region)

<i>H. pylori</i> status	<i>IL-1B</i> –511	Control (n = 169)	Cancer (n = 86)	χ^2	p Value	OR (95% CI)
<i>H. pylori</i> –	C/C	19	1	1.1	0.29*	NS
	T/T	17	3			
<i>H. pylori</i> +	C/C	15	18	13.3	<0.001†	2.7 (2.7–190.8)
	T/T	21	19			

*Cancer_{Hp–} T/T versus control_{Hp–} T/T: $\chi^2 = 1.1$, $p = 0.29$.†Cancer_{Hp+} C/C versus control_{Hp+} C/C: $\chi^2 = 13.3$, $p < 0.001$, OR 2.7, 95% CI 2.7–190.8.‡Cancer_{Hp+} T/T versus control_{Hp+} T/T: $\chi^2 = 6.1$, $p = 0.014$, OR 5, 95% CI 1.3–20.3.

in China, respectively. There are marked differences in phyla, figures, and hereditary diseases in these two regions.¹⁸ Their mean annual gastric cancer mortality rates have been estimated as 23.6 and 7.9/100 000 population.

In the present study, the *IL-1B* +3954 polymorphism did not show any correlation with gastric carcinogenesis, which echoes the finding of El-Omar and colleagues.⁴ *IL-1B* –511T/T was found in more than 23% of gastric cancer patients in both low prevalence and high prevalence regions as well in control subjects in the latter. This is compatible with previous reported in Shangdong,¹⁹ and the genotype frequency is much higher than that in non-cancer Caucasians.⁶ Furthermore, in the low prevalence region, the *IL-1B* –511T/T genotype was found to increase the risk of gastric cancer development by approximately 2.3-fold. While the findings in Guangdong province are similar to those reported in the literature, the picture is quite different in Shanxi province, a region of high prevalence for gastric cancer in China. In Shanxi, the frequency of the *IL-1B* –511T/T genotype was equally high in control and gastric cancer patients. The between group difference in the *IL-1B* –511 T/T genotype was insignificant. Although allele frequency did not demonstrate the significance of this proinflammatory genotype in gastric carcinogenesis, the high prevalence of this genotype even among non-cancer patients (23.0%) may in fact explain the high incidence of this malignancy. Therefore, individuals with proinflammatory genotypes who are also infected with *H. pylori* are expected to have severe hypochlorhydria, atrophy, and therefore a high risk of gastric cancer.

However, there seems to be some confusion about the –31 alleles in the present literature. El-Omar *et al* reported that –31C alleles increased the risk of gastric cancer (OR 1.9, 95% CI 1.5–2.6).⁴ In a Japanese study, Takagi *et al* found that *IL-1B* polymorphisms (*IL-1B* –511C/C and –31T/T) enhanced not only *IL-1 β* production but also *IL-8* production in the gastric body. They believed the above genotypes were associated with *H. pylori* infected gastric mucosal lesions (the development of atrophic gastritis).²⁰

In this study, we found that *IL-1B* –31C/C genotype frequency was higher in patients with gastric cancer than that in controls in the high prevalence region (90.0% *v* 78.0%; $\chi^2 = 5.0$, $p = 0.025$, OR 2.4, 95% CI 1.1–5.2). But this genotype frequency was higher in controls than in patients with gastric cancer in the low prevalence region. How do we explain these results? El-Omar *et al* considered the *IL-1B* –31C allele but not the T allele as a proinflammatory gene⁴ but the T allele is the first base of the TATA box in the *IL-1B* gene promoter. If the *IL-1B* –31C allele was considered as the proinflammatory allele, *IL-1 β* expression should be upregulated. However, mutation of T to C in the TATA box in the *IL-1B* gene promoter will result in downregulation of *IL-1 β* expression. Therefore, we believe that the *IL-1B* –31T allele may be a proinflammatory allele.

Unlike El-Omar's study however we did not find near complete linkage disequilibrium between the two loci. The

two genotypes produced almost independent risks for gastric carcinogenesis. A different mode of linkage between *IL-1B* –511 and –31 has also been reported in a study from Korea, indicating that there are multiple forms of linkages possible.¹⁶

We attempted to study the interaction between *H. pylori* infection and *IL-1B* genotype polymorphisms. In Guangdong province, T/T genotype individuals without *H. pylori* infection did not have an increased risk of gastric cancer. However, the opposite effect was apparent in C/C genotype individuals with *H. pylori* infection. Furthermore, if an infected individual also had the proinflammatory genotype *IL-1B* –511T/T, the risk of gastric carcinogenesis was dramatically elevated to over 17-fold. This finding suggests that the T/T genotype is not a carcinogenic factor without *H. pylori* infection, and C/C genotype individuals infected *H. pylori* also have a cancer risk. But a synergistic effect of the host genetic makeup (*IL-1B* –511 T allele) with *H. pylori* infection may underlie the process of carcinogenesis whereas it is obvious that both *H. pylori* and the T allele are necessary for the development of gastric cancer. In the high prevalence region, the risk of gastric cancer development in individuals with *H. pylori* infection was substantially increased. The cancer risk also existed in C/C genotype individuals with *H. pylori* infection. The presence of the *IL-1B* –511T/T genotype did not appear to further increase the risk of this population. The lack of synergistic effects of these two factors might be related to the low frequency of the C/C genotype in this population or the presence of another potent environmental factor, such as dietary habit. We must also point out that analysis of the interaction between *H. pylori* infection and *IL-1B* genotype polymorphisms in this study was limited by the differences in mean age and sex between the control subjects and patients with gastric cancer. As the process of *H. pylori* induced malignant transformation in the gastric mucosa takes decades to develop, an age and sex matched control group would provide a better comparison of the cancer risk.

In conclusion, this study confirms that *IL-1B* –511T/T genotypes are associated with an increased risk of gastric carcinoma in China. However, the effect of *IL-1B* polymorphism is less obvious in regions with a high prevalence of gastric cancer. The interaction between *IL-1B* polymorphism and other environmental factors such as *H. pylori* infection and dietary habit deserves further study.

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