GASTRIC CANCER

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

Z-R Zeng, P-J Hu, S Hu, R-P Pang, M-H Chen, M Ng, J J Y Sung

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-1β 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% vs 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.
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 erythematosis, diabetes mellitus, rheumatoid arthritis, or inflammatory bowel disease. Subjects with a family history of gastric cancer were also excluded. With the ethnic group of Han. None of these subjects had a history of systemic lupus erythematosis, diabetes mellitus, rheumatoid arthritis, or inflammatory bowel disease. Subjects with a family history of gastric cancer were also excluded.

Evaluation of \textit{H pylori} prevalence

\textit{H pylori} status of subjects was determined by an ELISA assay for anti-\textit{H pylori} IgG (BioChek Inc., USA). All analyses were done in duplicate and with an internal standard. Concentrations of \textit{H pylori} IgG were determined by reading at an optical density of 450 nm. Subjects were considered to be \textit{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:isoamyle 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 AvaI polymorphic site at position −31 of the IL-1B gene was amplified by PCR. The oligonucleotides 5’-AGAAGCTTCCACAAATCTC-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. Genotypes were designated as follows: TT, two bands of 100 and 52 bp; CT, three bands of 152, 100, and 52 bp; and TT, a single band of 150 bp.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic data and \textit{Helicobacter pylori} status of subjects in Guangdong (low prevalence region) and Shanxi (high prevalence region)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Guangdong (Low prevalence region)</td>
</tr>
<tr>
<td>No of subjects</td>
<td>192</td>
</tr>
<tr>
<td>Age (y) (mean (SD))</td>
<td>21.2 (1.35)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>119 (61.9%)</td>
</tr>
<tr>
<td>\textit{H pylori} positive (%)</td>
<td>67 (34.9%)</td>
</tr>
<tr>
<td>(sex adjusted rate)*</td>
<td>(36.0%)</td>
</tr>
</tbody>
</table>

A fragment containing the AvaI polymorphic site at position −31 of the IL-1B gene was amplified by PCR. The oligonucleotides 5’-AGAAGCTTCCACAAATCTC-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. Genotypes were designated as follows: TT, two bands of 100 and 52 bp; CT, three bands of 152, 100, and 52 bp; and TT, a single band of 150 bp.
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-1RN* 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-1RN* polymorphism was analysed by PCR that amplifies the tandem repeat regions, as described previously. The IL-RN polymorphism was analysed by PCR that amplifies the tandem repeat regions, as described previously.

### Statistical analysis

The sex difference in the two populations was adjusted according to the fifth population census of China. The Hardy-Weinberg equilibrium at individual loci was assessed by χ² statistics using the program SPSS (version 10.1, Chicago, Illinois, USA). Comparison of genotype frequencies between cases and controls was assessed by χ² 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.

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Guangdong (low prevalence region)</th>
<th>Shanxi (high prevalence region)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n = 192)</td>
<td>GC patients (n = 84)</td>
</tr>
<tr>
<td><strong>IL-1B</strong>−511</td>
<td>C/C</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>0</td>
</tr>
<tr>
<td><strong>IL-1B</strong>−31</td>
<td>C/C</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>0</td>
</tr>
</tbody>
</table>

*|CancerGuangdong versus controlGuangdong: χ²=6.7, p=0.010, OR 2.3, 95% CI 1.2–4.5.  
|CancerShanxi versus controlShanxi: χ²=7.0, p=0.008, OR 2.1, 95% CI 1.2–3.7.  

### Table 3  *IL-1RN* genotype frequencies in control subjects and gastric cancer (GC) patients in Guangdong (low prevalence region) and Shanxi (high prevalence region)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Guangdong (low prevalence region)</th>
<th>Shanxi (high prevalence region)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls (n = 192)</td>
<td>GC patients (n = 84)</td>
</tr>
<tr>
<td><strong>IL-1RN</strong></td>
<td>C/C</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>T/T</td>
<td>0</td>
</tr>
</tbody>
</table>

*|CancerGuangdong versus controlGuangdong: χ²=9.9, p=0.002, OR 2.5, 95% CI 1.4–4.4.  
|CancerShanxi versus controlShanxi: χ²=5.0, p=0.025, OR 2.4, 95% CI 1.1–5.2.
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%) (χ² = 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%; χ² = 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%; χ² = 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%; χ² = 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%; χ² = 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; χ² = 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%; χ² = 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%; χ² = 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 (χ² = 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 (χ² = 7.9, p = 0.005). The risk of cancer development was dramatically increased for the T/T genotype with H. pylori infection (χ² = 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 (χ² = 1.1, p = 0.29). H. pylori infection in the C/C genotype had a significantly raised risk of gastric cancer (χ² = 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 (χ² = 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 H. pylori 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. 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.8

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

### Table 5

<table>
<thead>
<tr>
<th>H pylori status</th>
<th>IL-1B-511</th>
<th>Control (n=192)</th>
<th>Cancer (n=84)</th>
<th>χ²</th>
<th>p Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H pylori− C/C</td>
<td>36</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>&lt;0.001</td>
<td>5.0 (1.5–16.3)</td>
</tr>
<tr>
<td>H pylori− T/T</td>
<td>20</td>
<td>3</td>
<td>0.7</td>
<td>0.4</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>H pylori+ C/C</td>
<td>51</td>
<td>4</td>
<td>16.9</td>
<td>5.0</td>
<td>0.005†</td>
<td>17.1 (3.8–76.4)</td>
</tr>
<tr>
<td>H pylori+ T/T</td>
<td>20</td>
<td>3</td>
<td>0.7</td>
<td>0.4</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

* Cancer vs control, T/T versus C/C, χ² = 0.7, p = 0.4.
† Cancer vs control, C/C versus T/T, χ² = 7.9, p = 0.005, OR 5.0, 95% CI 1.5–16.3.
‡ Cancer vs control, T/T versus C/C, χ² = 16.9, p<0.001, OR 17.1, 95% CI 3.8–76.4.
in China, respectively. There are marked differences in phyla, figures, and hereditary diseases in these two regions. 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. 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 Shandong, and the genotype frequency is much higher than that in non-cancer Caucasians. 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–511T/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 atrophic gastritis, 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). In a Japanese study, Takagi et al found that IL-1B polymorphisms (IL-1B−511C/C and −31T/T) enhanced not only IL-1β 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).

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% vs 78.0%; \(\chi^2 = 3.0, p = 0.025, OR 2.4, 95\% CI 1.1–5.2\)). 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 gene, but the T allele is the first base of the TATA box in the IL-1B gene promoter. If the IL-1B–31C allele were considered as the proinflammatory allele, IL-1β 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-1β 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.

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.

### Authors’ affiliations

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