ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
August 2019; 18(4):393-401.

Genetic Polymorphisms of CXCL8 (−251) Are Associated with the Susceptibility of Helicobacter pylori Infection Increased the Risk of Inflammation and Gastric Cancer in Thai Gastroduodenal Patients

Wongwarut Boonyanugomol1, Kamolchanok Rukseree1, Worrarat Kongkasame2, Pratis Palittapongarnpim3, Seung-Chul Baik4, and Mereerat Manwong5

1 Department of Sciences and Liberal Arts, Mahidol University, Amnat Charoen Campus, Amnat Charoen, Thailand
2 Unit of Endoscopy Medicine, Suppasittiprasong Hospital, Ubon Ratchathani, Thailand
3 Department of Microbiology, Faculty of Science, Mahidol University, Bangkok, Thailand
4 Department of Microbiology, Gyeongsang National Institute of Health Sciences, School of Medicine, Gyeongsang National University, Jinju, Republic of Korea
5 College of Medicine and Public Health, Ubon Ratchathani University, Ubon Ratchathani, Thailand

Received: 9 December 2018; Received in revised form: 31 March 2019; Accepted: 13 April 2019

ABSTRACT

CXC Chemokine Ligand 8 (CXCL8) plays an important role in gastric inflammation and in the progression of gastric cancer induced by Helicobacter pylori (H. pylori) infection. The association of CXCL8, CXC Chemokine Receptor 1 (CXCR1), and CXC Chemokine Receptor 2 (CXCR2) polymorphisms with H. pylori infection and gastric cancer progression needs to be investigated in a population within an enigma area consisting of multiple ethnicities, such as Thailand.

To analyze the relative risk of H. pylori infection and gastric cancer among Thai gastroduodenal patients, gene polymorphisms in CXCL8 (promoter region -251) and in CXCR1 and CXCR2 (receptors for CXCL8) were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and allele specific-PCR (AS-PCR). We also determined the presence of cytotoxin-associated gene A (cag-A) in Thai patients with H. pylori infection. Correlation between the CXCL8 (−251) polymorphism and CXCL8 gene expression was evaluated by quantitative reverse transcriptase-PCR (qRT-PCR).

We found a significant association between the T/A and A/A genotypes of CXCL8 (−251) with H. pylori infection. However, no significant correlation was found between the CXCR1 (+2607) and CXCR2 (+1208) gene polymorphisms with H. pylori infection among Thai gastroduodenal subjects. Within the H. pylori-infected group of Thai gastroduodenal patients, no significant differences in cag-A were observed. In addition, the A/A genotype of CXCL8 (−251) significantly correlated with the risk of gastric cancer and correlated with higher CXCL8 gene expression levels in Thai gastroduodenal patients.

These results suggest that CXCL8 (−251) polymorphisms are associated with H. pylori infection, an increased risk of stronger inflammatory responses, and gastric cancer in Thai gastroduodenal patients.

Keywords: CXC chemokine ligand 8; CXC chemokine receptor 1; CXC chemokine receptor 2; Gene polymorphism; Helicobacter pylori; Thai
INTRODUCTION

*Helicobacter pylori* (*H. pylori*) infection induces gastric mucosal inflammation and damage that are associated with various gastroduodenal diseases, such as chronic gastritis, peptic ulcer diseases (PUD), and gastric cancer.\(^1\) Thailand, located in Southeast Asia, contains multiple ethnic groups and cultures that likely contribute to genetic variation within the country.\(^2,3\) Although the prevalence of *H. pylori* infection is high in Thailand,\(^4\) but Thailand belongs to a low risk gastric cancer area called the Asian Enigma, as compared to East Asian countries such as Japan and Korea.\(^4\) Cytotoxin-associated gene A (CagA) of *H. pylori* plays an important role in the stimulation of CXC Chemokine Ligand 8 or CXCL8 (Interleukin-8, IL-8) production and is associated with increased risk of gastric cancer.\(^5\) However, previous data found that cagA-positive *H. pylori* infections associated with several different clinical outcomes of Thai gastroduodenal patients, with no significant difference between each clinical outcome.\(^6\) It was proposed that gastric carcinogenesis caused by *H. pylori* is a multifactorial process, which includes *H. pylori* strains, host genetic variations, host behavioral factors, and environmental factors.\(^7\) Therefore, a potential association between host genetic factors of Thai gastroduodenal patients and *H. pylori* infection should be investigated in order to elucidate the pathogenesis of *H. pylori* in Asian Enigma area.

CXCL8 acts as a chemotactic factor at infected sites in the early-phase of *H. pylori* infection, which is thought to be an initial step of gastric mucosal damage resulting from the production of reactive oxygen radicals.\(^8\) Several studies found that CXCL8 was associated with tumor progression, including cancer cell proliferation, angiogenesis, and tumor migration.\(^9-11\) Single nucleotide polymorphisms (SNPs) are single nucleotide variations of genomic DNA, which are the most common type of genetic polymorphism.\(^12\) Genetic polymorphisms may affect various cell functions, including gene transcription and translation that may have effects on disease susceptibility and pathological progression.\(^13-15\) Previous data showed that the heterozygous (T/A) and homozygous mutant (A/A) CXCL8 (-251) SNPs in the promoter region were associated with osteosarcoma in a Chinese population.\(^16\) In contrast, homozygous wild-type CXCL8 (-251 T/T) was associated with increased risk of non-small lung cancer in Tunisian patients.\(^17\) In *H. pylori* infections of Japanese patients, the CXCL8 (-251) SNP associated with the susceptibility to *H. pylori* infection and the risk of gastric cancer.\(^18\) These conflicting results may due to different ethnicities, organ susceptibility, or different causative agents of disease. Therefore, in the Asian Enigma region of Thailand, association of CXCL8 gene polymorphisms with the risk of *H. pylori* infection and gastric cancer is unknown and should be determined.

High affinity binding of CXCL8 to its specific receptors (CXC Chemokine Receptor 1; CXCR1 and CXC Chemokine Receptor 2; CXCR2) leads to several cellular responses.\(^19\) A possible association between the CXCR2 polymorphism +1208 C/T (located in the non-coding region) and the susceptibility to chronic inflammatory diseases such as systemic sclerosis and chronic obstructive pulmonary disease was previously described.\(^20,21\) Currently, no studies have investigated the association of CXCR1 and CXCR2 gene polymorphisms with the risk of *H. pylori* infection in gastroduodenal patients. Therefore, this study aimed to investigate the association of CXCL8 (-251), CXCR1 (+2607), and CXCR2 (+1208) gene polymorphisms with the risk of *H. pylori* infection and gastric cancer in Thai gastroduodenal patients. Simultaneously, we also evaluated the relationship between the CXCL8 (-251) polymorphisms and CXCL8 gene expression levels.

MATERIALS AND METHODS

Patients and Specimen Collection

Specimens were collected from Thai gastroduodenal patients undergoing gastroendoscopy at the Unit of Endoscopy Medicine, Suppasittiprasong Hospital, Ubon Ratchathani, which is located in Northeast Thailand. Clinical manifestations were classified as gastritis, PUD, or gastric cancer. A total of 80 patients, comprising 50 cases of gastritis, 20 of PUD, and 10 of gastric cancer, were diagnosed as being infected with *H. pylori* after testing positive with a rapid urease test kit (Pronto Dry, Gastrex, France) and with a 16SrRNA polymerase chain reaction (PCR) assay. *H. pylori*-uninfected patients were also included in the study, comprised of 30 cases of gastritis, 10 of PUD, and 4 of gastric cancer. Informed consent was obtained from each patient before specimen collection. This study was approved by the Human Ethics Committee of Mahidol University (COA.NO. MUCIRB 2016/157.0912).
Polymorphisms of CXCL8, CXCR1 and CXCR2 in Thai Gastroduodenal Patients

DNA Extraction and Genotyping

Genomic DNA was extracted from gastric tissue samples by grinding in a fitted pestle followed by purification using a DNA isolation kit according to the manufacturer’s instructions (DNAzol, Life Technologies Corporation, USA). After precipitation, DNA was dissolved in TE buffer and stored at -20°C until used.

The CXCL8 gene polymorphism at position-251 (rs4073) was detected by PCR-restriction fragment length polymorphism (PCR-RFLP) analysis. PCR was performed in a volume of 25 µL containing 50 ng of genomic DNA and 0.2 µM of each primer in a ready-to-use PCR master mix (One PCR Ultra™, BioHelix, Taiwan). PCR primer sequences and amplification conditions are shown in Table 1, which were based on a previously published protocol with slight modifications.22 CXCL8 (-251) PCR amplicons (108 bp) were subsequently digested with MspI (Thermo Scientific™, USA) at 37°C overnight. Digestion products were separated by electrophoresis on 5% agarose gels and visualized with a UV illuminator for the T/T, A/A, and T/A genotypes, the following fragment sizes were observed: 108 bp, 76 bp+32 bp, and 108 bp+76 bp+32 bp, respectively.

Gene polymorphisms of CXCR1 (+2607) (rs2234671) and CXCR2 (+1208) (rs1801032) were identified using allele-specific PCR (AS-PCR) (Table 1), as previously described.20 Briefly, genomic DNA was amplified in a total volume of 25 µL in two separate PCRs per polymorphism in which each reaction contained a generic antisense primer and one of two allele-specific sense primers. The cycling parameters of AS-PCR are presented in Table 1.

The prevalence of cagA was determined by PCR using cagA specific primers (PCR conditions are listed in Table 1), following a previously published report.23 PCRs were prepared in a final volume of 25 µL containing 100 ng of genomic DNA and 0.5 µM of primers in a ready-to-use PCR master mix (One PCR Ultra™, BioHelix, Taiwan). PCR products were analyzed by 1.5% agarose gel electrophoresis and were observed with a UV illuminator.

Table 1. Primer sequences used for determination of genetic polymorphisms of CXC chemokine ligand 8 (CXCL8), CXC chemokine receptor 1 (CXCR1), CXCR2 and PCR assay of Helicobacter pylori cytotoxin-associated gene A (cagA)

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Method</th>
<th>Primer Sequences</th>
<th>Conditions</th>
<th>Product Size</th>
</tr>
</thead>
</table>
| CXCL8 -251 T/A | PCR-RFLP   | F: 5’-TTCTAACACCTGCCACTCTTAG-3’
R: 5’-CTGAAGCTCCACAATTTGGTG-3’ | 94°C 5 min, 35 cycles of 94°C 30 sec, 60°C 30 sec, 72°C 30 sec, final extension of 72°C 7 min | 108 bp |
| CXCR1+2607 G/C | AS-PCR     | F: 5’-CCCAGGTGTGATCCAGAGAG-3’
R: 5’-CCCAGGTGTGATCCAGAGAC-3’ | 95°C 5 min, 35 cycles of 95°C 45 sec, 56°C 45 sec, 72°C 1 min, final extension of 72°C 8 min | 205 bp |
| CXCR2+1208 C/T | AS-PCR     | F: 5’-CCATTGTGTCAGCAAGATG-3’
F: 5’-CCATTGTGTCAGCAAGAC-3’
R: 5’-GTCTGTGAATAAGCTGCTATGA-3’ | 96°C 1 min, 5 cycles of 96°C 25 sec, 70°C 45 sec, and 72°C 25 sec, 21 cycles of 96°C 25 sec, 65°C 50 sec, and 72°C 30 sec, and 4 cycles of 96°C 30 sec, 55°C 60 sec, and 72°C 90 sec. | 627 bp |
| cagA          | PCR        | F: 5’-TGACCAAACAACACCACAAACCGAGAG-3’
R: 5’-CTCCCTTAATTGCGAGATC-3’ | 94°C 5 min, 35 cycles of 94°C 1 min, 52°C 1 min, 72°C 1 min, final extension of 72°C 7 min | 183 bp |

PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism
AS-PCR: allele-specific-polymerase chain reaction

Vol. 18, No. 4, August 2019
Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)
RNA Extraction and CXCL8 Gene Expression

RNA was extracted from gastric tissue samples using Trizol™ reagent (Life Technologies Corporation, USA) and grinding with a fitted pestle according to the manufacturer’s instructions. Precipitated RNA was dissolved in PCR-grade water. All RNA samples were quantified using a spectrophotometer and then stored at -80°C until used.

One microgram of RNA was reverse transcribed using random hexamers and the Super Script III First-Strand Synthesis System (Life Technologies Corporation, USA). Primers specific for CXCL8 (112 bp) and 18SrRNA (99 bp) were based on previously published sequences. CXCL8 and 18S rRNA gene primer sequences were (sense: 5′- ACTGAGAGTGATGAGAGTGAC-3′ and antisense: 5′-AACCCTCTGCAACCGATTTC-3′) and (sense: 5′-CGGCGACGACCCAATCGAAC-3′ and antisense: 5′-GAATCGAACCCTGATTCCCCGTC-3′), respectively. CXCL8 gene expression was quantified by quantitative reverse transcriptase-PCR (qRT-PCR) performed on a Light Cycler 96 thermal cycler (Roche Diagnostic, Germany). Briefly, 20 μL reactions containing 1x SYBR Green master mix (Roche Diagnostic, Germany), 1 μM of primers, and 1 μL of cDNA template were run under the following PCR conditions: 10 minutes at 95°C followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C. Duplicate reactions were run for each sample and CXCL8 gene expression values were normalized to the 18S rRNA housekeeping gene. Relative expression was analyzed by using 2^ΔΔCT method.

Statistical Analysis

Statistical analysis was performed using SPSS 10.0 software (SPSS Inc., Chicago, USA). To estimate the relationship between gene polymorphisms and the relative risk of H. pylori infection and gastric cancer, the odd ratios (OR) and their 95% confidence interval (95% CI) were calculated by logistic regression. The genotype frequency of each polymorphism was analyzed for deviation from the Hardy-Weinberg equilibrium by the Chi-square-test (Χ²). Relative CXCL8 gene expression levels are presented as mean±SEM. Student’s t-test was used to compare CXCL8 gene expression among the groups of gene polymorphisms. A p-value of ≤0.05 was considered statistically significant.

RESULTS

CXCL8 (-251), CXCR1 (+2607), and CXCR2 (+1208) Gene Polymorphisms in Thai Gastroduodenal Patients

As shown in Table 2, a high frequency of the homozygous wild-type CXCL8 (-251) (T/T) SNP was found in H. pylori-uninfected patients (77.27%). In contrast, the heterozygous (T/A) and homozygous mutant (A/A) SNPs of CXCL8 (-251) were significantly associated with H. pylori-infected patients (ORs=11.60, 95% CI: 4.22-31.94, p<0.001 and ORs=6.48, 95% CI: 1.91-22.01, p=0.003, respectively), indicating that the T/A and A/A genotypes of CXCL8 (-251) are associated with increased risk of H. pylori infection in the Thai population. Similarly, the A allele of CXCL8 (-251) showed a significant association with susceptibility to H. pylori infection (ORs=4.66, 95% CI: 2.43-8.94, p<0.001). The distribution of the CXCL8 genotypes was consistent with the Hardy-Weinberg equilibrium in H. pylori-infected patients (X²=0.502, p=0.479), but not in H. pylori-uninfected patients (X²=10.579, p=0.001).

We also determined frequencies of the CXCR1 (+2607) and CXCR2 (+1208) polymorphisms in Thai gastroduodenal patients, as shown in Table 2. Homozygous wild-type CXCR1 (+2607; G/G) and CXCR2 (+1208; C/C) were most frequently found in Thai gastroduodenal patients, both with and without H. pylori infection, whereas heterozygous CXCR1 (+2607; G/C) and CXCR2 (+1208; C/T) were rarely detected in either group. However, homozygous mutant CXCR1 (+2607; C/C) and CXCR2 (+1208; T/T) were not detected. These findings indicate that gene polymorphisms in CXCR1 or CXCR2 were not associated with H. pylori infection in Thai gastroduodenal patients. Likewise, no significant differences in the rate of the G (CXCR1 +2607) and C (CXCR2 +1208) alleles between the two populations were observed (p>0.05). The distribution of CXCR1 (+2607) and CXCR2 (+1208) polymorphisms was consistent with the Hardy-Weinberg equilibrium, both in H. pylori-infected patients (CXCR1: Χ²=0.222, p=0.638; CXCR2: Χ²=0.053, p=0.819) and H. pylori-uninfected patients (CXCR1: Χ²=0.236, p=0.627; CXCR2: Χ²=0.100, p=0.752).

The cagA gene was detected in 63 of 80 H. pylori-infected samples from Thai gastroduodenal patients (Table 3). The cagA gene was detected in 38 samples from uninfected patients...
Polymorphisms of CXCL8, CXCR1 and CXCR2 in Thai Gastroduodenal Patients

(76%), 17 samples (85%), and 8 samples (80%) from gastritis, PUD, and gastric cancer patients, respectively. However, significant differences in cagA detection were not found among these three clinical outcomes of Thai subjects.

Among the three clinical presentations of H. pylori-infected Thai gastroduodenal subjects shown in Table 4, the homozygous mutant of CXCL8 (-251) (A/A) had a protective effect against gastritis (ORs 0.27, 95% CI 0.09-0.85, p=0.026) but was not associated with the PUD group (ORs 0.64, 95% CI 0.16-2.52, p=0.521). Moreover, we found that the A/A genotype correlated with a 16-fold increase in the risk of gastric cancer (ORs 15.82, 95% CI 3.45-72.52, p<0.001) as compared with gastric cancer subjects carrying the T/T or T/A genotypes.

Table 2. Genotype and allele frequencies of CXC Chemokine Ligand 8 (CXCL8), CXC Chemokine Receptor 1 (CXCR1) and CXC Chemokine Receptor 2 (CXCR2) in Thai patients with gastroduodenal diseases

<table>
<thead>
<tr>
<th>Gene polymorphisms</th>
<th>H. pylori Positive (n=80)</th>
<th>H. pylori Negative (n=44)</th>
<th>ORs (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL8 (-251)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>21 (26.25%)</td>
<td>34 (77.27%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>T/A</td>
<td>43 (53.75%)</td>
<td>6 (13.63%)</td>
<td>11.60 (4.22-31.94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A/A</td>
<td>16 (20.00%)</td>
<td>4 (9.10%)</td>
<td>6.48 (1.91-22.01)</td>
<td>0.003</td>
</tr>
<tr>
<td>T allele</td>
<td>85 (53.13%)</td>
<td>74 (84.10%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A allele</td>
<td>16 (20.00%)</td>
<td>4 (9.10%)</td>
<td>6.48 (1.91-22.01)</td>
<td>0.003</td>
</tr>
<tr>
<td>CXCR1 (+2607)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>72 (90%)</td>
<td>38 (86.36%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>G/C</td>
<td>8 (10%)</td>
<td>6 (13.64%)</td>
<td>0.70 (0.23-2.18)</td>
<td>0.542</td>
</tr>
<tr>
<td>C/C</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G allele</td>
<td>152 (95%)</td>
<td>82 (93.18%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C allele</td>
<td>8 (5%)</td>
<td>6 (6.82%)</td>
<td>0.72 (0.24-2.14)</td>
<td>0.554</td>
</tr>
<tr>
<td>CXCR2 (+1208)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>76 (95%)</td>
<td>40 (90.91%)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>4 (5%)</td>
<td>4 (9.09%)</td>
<td>0.53 (0.13-2.22)</td>
<td>0.382</td>
</tr>
<tr>
<td>T/T</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C allele</td>
<td>156 (97.50%)</td>
<td>84 (95.45%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T allele</td>
<td>4 (2.50%)</td>
<td>4 (4.55%)</td>
<td>0.54 (0.13-2.21)</td>
<td>0.390</td>
</tr>
</tbody>
</table>

Table 3. Prevalence of H. pylori cytotoxin-associated gene A (cagA) gene in Thai patients with gastroduodenal diseases

<table>
<thead>
<tr>
<th>cagA status</th>
<th>H. pylori-infected subjects (n=80)</th>
<th>PUD (n=20)</th>
<th>Gastric cancer (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cagA positive</td>
<td>38 (76%)</td>
<td>17 (85%)</td>
<td>8 (80%)</td>
</tr>
<tr>
<td>cagA negative</td>
<td>12 (24%)</td>
<td>3 (15%)</td>
<td>2 (20%)</td>
</tr>
</tbody>
</table>

PUD: Peptic ulcer diseases

Table 4. Association of CXC chemokine ligand 8 (CXCL8-251) polymorphisms in Thai patients with three clinical outcomes of Helicobacter pylori (H. pylori) infection

<table>
<thead>
<tr>
<th>Clinical outcomes (n=80)</th>
<th>CXCL8 (-251) polymorphisms</th>
<th>A/A</th>
<th>T/T/TA</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastritis (n=50)</td>
<td>6 (12%)</td>
<td>44 (88%)</td>
<td>0.27 (0.09-0.85)</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Peptic ulcer diseases (n=20)</td>
<td>3 (15%)</td>
<td>17 (85%)</td>
<td>0.64 (0.16-2.52)</td>
<td>0.521</td>
<td></td>
</tr>
<tr>
<td>Gastric cancer (n=10)</td>
<td>7 (70%)</td>
<td>3 (30%)</td>
<td>15.82 (3.45-72.52)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>
Effect of CXCL8 (-251) Polymorphism on CXCL8 Gene Expression in Thai Patients with H. pylori Infection

As shown above, the A/A genotype of the CXCL8 (-251) polymorphism significantly associated with an increased risk of gastric cancer. We subsequently determined the impact of the CXCL8 (-251) polymorphism on CXCL8 gene expression in Thai patients infected with H. pylori using qRT-PCR. We found equal expression levels of CXCL8 containing either the homozygous wild-type (T/T) or heterozygous (T/A) polymorphisms in Thai gastric tissues. Interestingly, a significant increase of CXCL8 gene expression was observed with the homozygous mutant (A/A) polymorphism, as compared to homozygous wild-type (T/T) and heterozygous (T/A) (Figure 1).

DISCUSSION

In the present study, we demonstrated that the T/A and A/A genotypes at the CXCL8 (-251) position were associated with an increased risk of H. pylori infection in Thai gastroduodenal patients. Consistent with the allele frequencies, subjects with the CXCL8 (-251) A allele tended to be at increased risk for H. pylori infection. Our observations are consistent with results from studies conducted in Bangladesh and Brazil, which demonstrated that the presence of the T/A or A/A genotype at the CXCL8 (-251) position may be a risk factor for H. pylori infection, whereas the T/T genotype may act as a protective factor against H. pylori infection. However, our data suggests that genetic variations across ethnicities may influence susceptibility and outcomes of H. pylori infections. The possible link between CXCL8 gene polymorphisms and H. pylori infection needs to be further studied. We hypothesize that CXCL8 gene polymorphisms may alter molecular signaling, which in turn may affect certain adhesion molecules leading to the generation of local niches for H. pylori colonization. However, the connection between molecular signaling, CXCL8 polymorphisms, and H. pylori colonization should be further elucidated.

CXCL8 is a well-known potent pro-inflammatory cytokine that exerts its effects by binding to the G protein-coupled receptors CXCR1 and CXCR2. CXCR1 is specific for CXCL8, whereas CXCR2 specifically binds to CXCL8 as well as to other CXC chemokines, including the epithelial cell-derived neutrophil-activating protein 7 (ENA-78), growth-related oncogene alpha (GRO-α), GRO-β, GRO-γ, and lipopolysaccharide-induced CXC chemokine. Interaction between CXCL8 and CXCR1/2 in the tumor microenvironment is involved in cancer progression and metastasis. Several studies have shown that CXCR1 or CXCR2 gene polymorphisms are
Polymorphisms of CXCL8, CXCRI and CXCR2 in Thai Gastroduodenal Patients

significantly associated with many diseases. For example, the C allele of CXCRI (+2607) is associated with significantly increased susceptibility to acute pylonephritis in childhood and a genetic polymorphism of the CXCR2 gene (+1208 T/T) is associated with systemic sclerosis. However, there has been no evidence showing that CXCRI and CXCR2 gene polymorphisms are linked to gastroduodenal diseases, especially in association with H. pylori infection. Our results indicated that gene polymorphisms of CXCRI and CXCR2 may not have any impact on risks of H. pylori infection or gastric carcinogenesis in Thai populations. However, these findings may be due to a small sample size. Subsequent investigation with a larger sample size is warranted to clarify the association of CXCRI or CXCR2 with H. pylori infection in Thai populations. We also suggest that additional studies with individuals of different ethnicities would help to address these issues.

CXCL8 production during H. pylori infection is caused by several virulence-associated components, particularly cagA, which is considered to be the major virulence gene involved in gastric inflammation and carcinogenesis. Our study found no association between the cagA gene and three clinical manifestations of Thai gastroduodenal subjects. Our data support previous observations made by Chomvarin et al who reported that the H. pylori cagA and cagE genes were detected at higher rates but a statistically significant association with clinical outcomes in Thai dyspeptic patients was not observed. However, specific features of host–pathogen interactions can generate variations in clinical outcomes after exposure to H. pylori; thus, we suggest that non-bacterial factors are also involved in determining clinical outcomes. We hypothesize that host genetic factors may play an important role in the pathogenesis of H. pylori infection in Thai populations, particularly in the inflammatory level mediated gastric carcinogenesis.

Additionally, the A/A genotype (CXCL8 -251) correlated with increased risk of gastric cancer, while acting as protective factor against gastritis in Thai subjects. Although Thailand is clustered in the Asian Enigma area where gastric cancer epidemiology is low, the A/A genotype was found to be associated with gastric cancer, which is similar to East Asian countries that have a high risk of gastric cancer, as demonstrated in previous studies of Korean and Japanese populations. Additionally, previous studies have shown that the CXCL8 (-251 A/A) genotype was also associated with an increased risk of other cancers, such as prostate cancer and Kaposi’s sarcoma. However, the A/A genotype was found to associate with gastric cancer, but the total number of gastric cancer cases was low in this study due to the very low prevalence of gastric cancer in Thailand. We suggest that CXCL8 polymorphisms may play an important role in gastric cancer development involving H. pylori infection in Thai subjects, but a large gastric cancer study of Thai populations is still required to confirm our study.

To understand the association between CXCL8 gene polymorphisms and CXCL8 gene expression, we determined the impact of each CXCL8 (-251) genotype on CXCL8 gene expression by qRT-PCR. It is noteworthy that CXCL8 (-251) polymorphisms have been tentatively associated with increased CXCL8 gene expression. Although the homozygous mutant (A/A) was found at a low frequency in the H. pylori-infected Thai population, but high levels of CXCL8 gene expression distribution were found among this genotype. Our results were similar to previous data of Chang et al that showed that CXCL8 production levels were low in Korean subjects carrying either the T/T or T/A genotype but were markedly high in subjects with the A/A genotype. Our findings are also consistent with data of Ohyauchi et al and Taguchi et al that showed that the A/A genotype of CXCL8 (-251) correlated with increased levels of CXCL8 production and neutrophil infiltration as compared to the T/T genotype, which may be related to severity of inflammation. Additionally, our data could be explained by a previously published report that showed that the CXCL8 (-251A) genotype correlated with enhanced promoter activity in response to IL-1β or tumor necrosis factor α (TNF-α) in an in vitro assay, which demonstrated an association between this allele and increased CXCL8 gene transcription. CXCL8 acts as a chemoattractant that recruits neutrophils and lymphocytes into infected tissue to promote the initiation and amplification of immunological responses. Subsequently, increased levels of CXCL8 may induce development of cancer cells via several processes such as angiogenesis, cell proliferation, and cancer cell progression. Therefore, we conclude that the homozygous mutant (A/A) at the CXCL8 (-251) position may be associated with heightened CXCL8 gene expression and may be linked to gastric
carcinogenesis in Thai populations, especially when *H. pylori* infections are involved.

In summary, we demonstrated that the heterozygous (T/A) and homozygous mutant (A/A) genotypes of CXCL8 (−251) correlated with increased susceptibility to *H. pylori* infection; moreover, the A/A genotype acts as a risk factor against severity of inflammation and gastric cancer induced by *H. pylori* infection in Thai populations. However, our pilot study consisted of 80 cases and 44 controls and therefore must be considered preliminary. To clarify *H. pylori* pathogenesis, studies of polymorphisms in other cytokine genes and other genetic factors should be conducted in populations of the Asian Enigma area.

**ACKNOWLEDGMENTS**

This study was supported by Thailand Research Fund (MRG5980069).

**REFERENCES**

Polymorphisms of CXCL8, CXCRI and CXCRII in Thai Gastrodoudenal Patients


Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)