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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

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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 (*cagA*) 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 *cagA* 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

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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. MU-CIRB 2016/157.0912).

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 *Mun*I (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*)

Polymorphisms	Method	Primer Sequences	Conditions	Product Size
CXCL8 -251 T/A	PCR-RFLP	F: 5'-TTCTAACACCTGCCACTCTAG-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'-CCCAGGTGATCCAGGAGAG-3' F: 5'-CCCAGGTGATCCAGGAGAC-3' R: 5'-TCAGAGGGTTGGAAGAGACATT-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'-CCATTGTGGTCACAGGAAGT-3' F: 5'-CCATTGTGGTCACAGGAAGC-3' R: 5'-GTCTTGTGAATAAGCTGCTATGA-3'	96°C 1 min, 5 cycles of 96°C 25 sec, 70° 45 sec, and 72°C 25 sec, 21cycles 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' -TTGACCAACAACCACAAACCGAAG-3' R: 5' -CTTCCCTTAATTGCGAGATTCC-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

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.[24,25] *CXCL8* and *18S rRNA* gene primer sequences were (sense: 5′-ACTGAGAGTGATTGAGAGTGGAC-3′ and antisense: 5′-AACCCTCTGCACCCAGTTTTC-3′) and (sense: 5′-CGGCGACGACCCATTCGAAC-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 *18SsRNA* housekeeping gene. Relative expression was analyzed by using $2^{-\Delta\Delta CT}$ 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 (X^2). 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 $\leq$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 ($X^2=0.502$, $p=0.479$), but not in *H. pylori*-uninfected patients ($X^2=10.579$, $p=0.001$).

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*: $X^2=0.222$, $p=0.638$; *CXCR2*: $X^2=0.053$, $p=0.819$) and *H. pylori*-uninfected patients (*CXCR1*: $X^2=0.236$, $p=0.627$; *CXCR2*: $X^2=0.100$, $p=0.752$).

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

(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

Gene polymorphisms	*H. pylori* Positive (n=80)	*H. pylori* Negative (n=44)	ORs (95% CI)	*p*-value
***CXCL8* (-251)**				
T/T	21 (26.25%)	34 (77.27%)	1.00	
T/A	43 (53.75%)	6 (13.63%)	11.60 (4.22-31.94)	<0.001
A/A	16 (20.00%)	4 (9.10%)	6.48 (1.91-22.01)	0.003
T allele	85 (53.13%)	74 (84.10%)		
A allele	75 (46.87%)	14 (15.90%)	4.66 (2.43-8.94)	<0.001
***CXCR1* (+2607)**				
G/G	72 (90%)	38 (86.36%)	1.00	
G/C	8 (10%)	6 (13.64%)	0.70 (0.23-2.18)	0.542
C/C	0 (0%)	0 (0%)	-	-
G allele	152 (95%)	82 (93.18%)		
C allele	8 (5%)	6 (6.82%)	0.72 (0.24-2.14)	0.554
***CXCR2* (+1208)**				
C/C	76 (95%)	40 (90.91%)	1.00	
C/T	4 (5%)	4 (9.09%)	0.53 (0.13-2.22)	0.382
T/T	0 (0%)	0 (0%)	-	-
C allele	156 (97.50%)	84 (95.45%)		
T allele	4 (2.50%)	4 (4.55%)	0.54 (0.13-2.21)	0.390

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

cagA status	*H. pylori*-infected subjects (n=80)		
	Gastritis (n=50)	PUD (n=20)	Gastric cancer (n=10)
cagA positive	38 (76%)	17 (85%)	8 (80%)
cagA negative	12 (24%)	3 (15%)	2 (20%)

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

Clinical outcomes (n=80)	*CXCL8* (-251) polymorphisms			
	A/A	TT/TA	OR (95%CI)	*p*-value
Gastritis (n=50)	6 (12%)	44 (88%)	0.27 (0.09-0.85)	0.026
Peptic ulcer diseases (n=20)	3 (15%)	17 (85%)	0.64 (0.16-2.52)	0.521
Gastric cancer (n=10)	7 (70%)	3 (30%)	15.82 (3.45-72.52)	<0.001

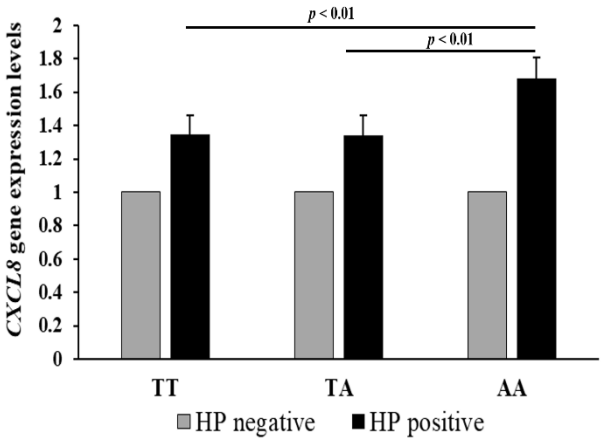

Figure 1. Relative expression levels of CXC chemokine ligand 8 (*CXCL8*) gene measured by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) among the TT, TA, and AA genotypes of the *CXCL8* (−251) polymorphism in the *Helicobacter pylori*-infected group. Data are presented as mean ± SEM. Significantly increased *CXCL8* gene expression levels were detected in samples containing the AA genotype as compared with the TT and TA genotypes. P values determined by student *t*-test were confirmed to be significant ($p<0.01$).

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 of *H. pylori* infection, whereas the T/T genotype may act as a protective factor against *H. pylori* infection.[26,27] 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.[28] 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 78 (ENA-78), growth-related oncogene alpha (GRO-α), GRO-β, GRO-γ, and lipopolysaccharide-induced CXC chemokine.[29,30] Interaction between CXCL8 and CXCR1/2 in the tumor microenvironment is involved in cancer progression and metastasis.[31,32] Several studies have shown that *CXCR1* or *CXCR2* gene polymorphisms are

significantly associated with many diseases. For example, the C allele of *CXCR1* (+2607) is associated with significantly increased susceptibility to acute pyelonephritis in childhood [33] and a genetic polymorphism of the *CXCR2* gene (+1208 T/T) is associated with systemic sclerosis.[20] However, there has been no evidence showing that *CXCR1* and *CXCR2* gene polymorphisms are linked to gastroduodenal diseases, especially in association with *H. pylori* infection. Our results indicated that gene polymorphisms of *CXCR1* 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 *CXCR1* 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.[34] 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.[6] 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.[18,35] 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.[36,37] 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.[35] 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.[18,22] 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.[18] CXCL8 acts as a chemoattractant that recruits neutrophils and lymphocytes into infected tissue to promote the initiation and amplification of immunological responses.[38] Subsequently, increased levels of CXCL8 may induce development of cancer cells via several processes such as angiogenesis, cell proliferation, and cancer cell progression.[38] 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.

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REFERENCES

1. Ruggiero P. Helicobacter pylori and inflammation. Curr Pharm Des 2010; 16(38):4225-36.
2. Kutanan W SS, Kamlao A, Kampuansai J. Mitochondrial DNA-HVR1 Variation Reveals Genetic Heterogeneity in Thai-Isan Peoples from the Lower Region of Northeastern Thailand. Advances in Anthropology 2014; 4(1):7-12.
3. Suyasunanont U, Nakkuntod M, Mirasena S. Mitochondrial DNA control region analysis of three ethnic populations in lower Northern part of Thailand. Genet Mol Res 2017; 16(3).
4. Fock KM, Ang TL. Epidemiology of Helicobacter pylori infection and gastric cancer in Asia. J Gastroenterol Hepatol 2010; 25(3):479-86.
5. Fazeli Z, Alebouyeh M, Rezaei Tavirani M, Azimirad M, Yadegar A. Helicobacter pylori CagA induced interleukin-8 secretion in gastric epithelial cells. Gastroenterol Hepatol Bed Bench 2016; 9(Suppl1):S42-S6.
6. Chomvarin C, Namwat W, Chaicumpar K, Mairiang P, Sangchan A, Sripa B, et al. Prevalence of Helicobacter pylori vacA, cagA, cagE, iceA and babA2 genotypes in Thai dyspeptic patients. Int J Infect Dis 2008; 12(1):30-6.
7. Fuccio L, Eusebi LH, Bazzoli F. Gastric cancer, Helicobacter pylori infection and other risk factors. World J Gastrointest Oncol 2010; 2(9):342-7.
8. Craig PM, Territo MC, Karnes WE, Walsh JH. Helicobacter pylori secretes a chemotactic factor for monocytes and neutrophils. Gut 1992; 33(8):1020-3.
9. Schadendorf D, Moller A, Algermissen B, Worm M, Sticherling M, Czarnetzki BM. IL-8 produced by human malignant melanoma cells in vitro is an essential autocrine growth factor. J Immunol 1993; 151(5):2667-75.
10. Koch AE, Polverini PJ, Kunkel SL, Harlow LA, DiPietro LA, Elner VM, et al. Interleukin-8 as a macrophage-derived mediator of angiogenesis. Science 1992; 258(5089):1798-801.
11. Wang JM, Taraboletti G, Matsushima K, Van Damme J, Mantovani A. Induction of haptotactic migration of melanoma cells by neutrophil activating protein/interleukin-8. Biochem Biophys Res Commun 1990; 169(1):165-70.
12. Vignal A, Milan D, SanCristobal M, Eggen A. A review on SNP and other types of molecular markers and their use in animal genetics. Genet Sel Evol 2002; 34(3):275-305.
13. Chien MH, Yeh CB, Li YC, Wei LH, Chang JH, Peng YT, et al. Relationship of interleukin-8 gene polymorphisms with hepatocellular carcinoma susceptibility and pathological development. J Surg Oncol 2011; 104(7):798-803.
14. Fu JW, Wang KW, Qi ST. Role of IL-8 gene polymorphisms in glioma development in a Chinese population. Genet Mol Res 2016; 15(3).
15. Bidwell JL, Wood NA, Morse HR, Olomolaiye OO, Laundy GJ. Human cytokine gene nucleotide sequence alignments, 1998. Eur J Immunogenet 1998; 25(2-3):83-265.
16. Chen Y, Yang Y, Liu S, Zhu S, Jiang H, Ding J. Association between interleukin 8 -251 A/T and +781 C/T polymorphisms and osteosarcoma risk in Chinese population: a case-control study. Tumour Biol 2016; 37(5):6191-6.
17. Rafrafi A, Chahed B, Kaabachi S, Kaabachi W, Maalmi H, Hamzaoui K, et al. Association of IL-8 gene polymorphisms with non small cell lung cancer in Tunisia: A case control study. Hum Immunol 2013; 74(10):1368-74.
18. Ohyauchi M, Imatani A, Yonechi M, Asano N, Miura A, Iijima K, et al. The polymorphism interleukin 8 -251 A/T influences the susceptibility of Helicobacter pylori related gastric diseases in the Japanese population. Gut 2005; 54(3):330-5.

19. Lee J, Horuk R, Rice GC, Bennett GL, Camerato T, Wood WI. Characterization of two high affinity human interleukin-8 receptors. J Biol Chem 1992; 267(23):16283-7.
20. Renzoni E, Lympany P, Sestini P, Pantelidis P, Wells A, Black C, et al. Distribution of novel polymorphisms of the interleukin-8 and CXC receptor 1 and 2 genes in systemic sclerosis and cryptogenic fibrosing alveolitis. Arthritis Rheum 2000; 43(7):1633-40.
21. Barnes PJ. Genetics and pulmonary medicine. 9. Molecular genetics of chronic obstructive pulmonary disease. Thorax 1999; 54(3):245-52.
22. Taguchi A, Ohmiya N, Shirai K, Mabuchi N, Itoh A, Hirooka Y, et al. Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. Cancer Epidemiol Biomarkers Prev 2005; 14(11 Pt 1):2487-93.
23. Akopyants NS, Clifton SW, Kersulyte D, Crabtree JE, Youree BE, Reece CA, et al. Analyses of the cag pathogenicity island of Helicobacter pylori. Mol Microbiol 1998; 28(1):37-53.
24. Takahashi A, de Andres MC, Hashimoto K, Itoi E, Oreffo RO. Epigenetic regulation of interleukin-8, an inflammatory chemokine, in osteoarthritis. Osteoarthritis Cartilage 2015; 23(11):1946-54.
25. Thomasson M, Hedman H, Junttila TT, Elenius K, Ljungberg B, Henriksson R. ErbB4 is downregulated in renal cell carcinoma--a quantitative RT-PCR and immunohistochemical analysis of the epidermal growth factor receptor family. Acta Oncol 2004; 43(5):453-9.
26. Ritu Saha MAI, Abu Naser Ibne Sattar, Sharmeen Ahmed. The Interleukin-8 -251 A Allele is Associated with Increased Risk of Different Gastroduodenal Diseases in H. pylori Infected Bangladeshi Patients. American Journal of Infectious Diseases and Microbiology 2016; 4(5):102-6.
27. Caleman Neto A, Rasmussen LT, de Labio RW, de Queiroz VF, Smith Mde A, Viani GA, et al. Gene polymorphism of interleukin 1 and 8 in chronic gastritis patients infected with Helicobacter pylori. J Venom Anim Toxins Incl Trop Dis 2014; 20:17.
28. Morris SW, Nelson N, Valentine MB, Shapiro DN, Look AT, Kozlosky CJ, et al. Assignment of the genes encoding human interleukin-8 receptor types 1 and 2 and an interleukin-8 receptor pseudogene to chromosome 2q35. Genomics 1992; 14(3):685-91.
29. Ahuja SK, Murphy PM. The CXC chemokines growth-regulated oncogene (GRO) alpha, GRObeta, GROgamma, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. J Biol Chem 1996; 271(34):20545-50.
30. Murphy PM. Neutrophil receptors for interleukin-8 and related CXC chemokines. Semin Hematol 1997; 34(4):311-8.
31. Ha H, Debnath B, Neamati N. Role of the CXCL8-CXCR1/2 Axis in Cancer and Inflammatory Diseases. Theranostics 2017; 7(6):1543-88.
32. Sharma B, Singh S, Varney ML, Singh RK. Targeting CXCR1/CXCR2 receptor antagonism in malignant melanoma. Expert Opin Ther Targets 2010; 14(4):435-42.
33. Javor J, Bucova M, Cervenova O, Kralinsky K, Sadova E, Suchankova M, et al. Genetic variations of interleukin-8, CXCR1 and CXCR2 genes and risk of acute pyelonephritis in children. Int J Immunogenet 2012; 39(4):338-45.
34. Schneider N, Krishna U, Romero-Gallo J, Israel DA, Piazuelo MB, Camargo MC, et al. Role of Helicobacter pylori CagA molecular variations in induction of host phenotypes with carcinogenic potential. J Infect Dis 2009; 199(8):1218-21.
35. Chang YW, Oh CH, Kim JW, Lee JW, Park MJ, Shim JJ, et al. Combination of Helicobacter pylori infection and the interleukin 8 -251 T > A polymorphism, but not the mannose-binding lectin 2 codon 54 G > A polymorphism, might be a risk factor of gastric cancer. BMC Cancer 2017; 17(1):388.
36. McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A, et al. Influence of cytokine gene polymorphisms on the development of prostate cancer. Cancer Res 2002; 62(12):3369-72.
37. van der Kuyl AC, Polstra AM, Weverling GJ, Zorgdrager F, van den Burg R, Cornelissen M. An IL-8 gene promoter polymorphism is associated with the risk of the development of AIDS-related Kaposi's sarcoma: a case-control study. AIDS 2004; 18(8):1206-8.
38. Xie K. Interleukin-8 and human cancer biology. Cytokine Growth Factor Rev 2001; 12(4):375-91.