Association of the myeloperoxidase-468G→A polymorphism with gastric inflammation and duodenal ulcer risk

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INTRODUCTION

The discovery of Helicobacter pylori (H pylori) heralded a breakthrough in the field of gastroenterology. It is a well-recognized pathogen that chronically infects more than 50% of the world’s population. Infection with the bacterium regularly leads to chronic gastritis. A subset of infected patients develops duodenal ulcer (DU), gastric ulcer, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma (MAL-Toma)\[1\]-[4]. The course of disease is affected by bacterial virulence factors, as well as genetic predisposition and environmental factors of the hosts.

It has been suggested that phenotypic or genotypic differences of the cag pathogenicity island, vacA, iceA and babA among bacterial strains may account for the development of severe diseases\[5\]-[8]. Early studies indicated that the cagA gene/CagA protein was a marker for more severe diseases because it was more frequently associated with strains isolated from patients with ulcer diseases or gastric adenocarcinoma\[9\]-[10]. However, the predominant strain of H pylori that circulates in Asian countries is a cagA-positive, vacA s1 and babA2 genotype, unrelated to clinical outcomes\[11\]-[12]. Bacterial virulent factors have so far failed to explain why the ulcer or gastric cancer phenotype develops or gastric cancer phenotype develops\[12\].\[13\]. Recently, attention has been focused on the involvement of host factors that determine susceptibility to H pylori-associated diseases, such as gender, blood groups, gastric acid physiology, human leukocyte antigen and genetic polymorphisms\[15\]-[18].

It is well known that H pylori infection is characterized by extensive infiltration of neutrophils. Neutrophils may...
generate superoxide anion (O$_2^-$), hydroxyl radical (·OH) and nonradical oxidants, such as hypochlorous acid (HOCl), to kill invading micro-organisms\[^{16-18}\]. Myeloperoxidase is an important enzyme of neutrophils. It is the key enzyme for the formation of HOCl from H$_2$O$_2$ in the presence of chloride ions. HOCl is a potent oxidant known to have several cytotoxic effects on bacterial cells. The integrity of bacterial cell membrane may be violated by the oxidation of membrane proteins\[^{17}\]. Additionally, activated neutrophils and monocytes can also generate cytoxic chloramines, tyroxyl radicals, and ·OH via an myeloperoxidase-dependent pathway\[^{16,18}\].

The inter-individual variations in myeloperoxidase activity of neutrophils are genetically determined\[^{19}\]. A diallelic polymorphism at the promoter region of myeloperoxidase gene (-463 bp) was observed, and is related to the transcription activity of this gene\[^{20}\]. The G allele is the wild type with normal expression, while the A allele is a low-expression allele\[^{20}\]. Recently, Roe et al\[^{21}\] disclosed that myeloperoxidase genotype critically determines the pathogenesis of atrophic gastritis subsequent to H pylori infection. However, no data exist regarding the relationships between myeloperoxidase genotype and peptic ulcer disease. The purposes of this study were to elucidate the relations between the myeloperoxidase -463G→A polymorphism and the development of DU, and to investigate the impacts of this host genetic polymorphism on the histopathological features of H pylori-related gastritis.

**MATERIALS AND METHODS**

**Subjects**

One hundred and fifteen consecutive unrelated Taiwanese with DU, who attended the Kaohsiung Veterans General Hospital, were included in this study. The diagnosis of DU was confirmed by endoscopic examination. An ulcer was defined as a circumscribed mucosal break 5 mm or more in diameter, with a well-defined ulcer crater. The size of ulceration was measured by opening a pair of biopsy forceps of known span in front of the ulcer. One hundred and eighty-five consecutive ethically matched asymptomatic healthy volunteers without active or past DU history served as healthy controls (HCs), and their endoscopic findings were all normal or showed gastritis only. The asymptomatic controls were enrolled from our health examination clinics. For them, panendoscopy was a routine examination of the general health checkup because the gastric cancer incidence was high in our country. To minimize ethnic bias, all patients and controls were Han Chinese; aboriginal and alien populations were excluded. Exclusion criteria for both groups included (1) history of esophageal or gastric ulcer, (2) previous history of anti-H pylori therapy, (3) use of non-steroidal anti-inflammatory drug or proton pump inhibitors within one month of endoscopy, (4) associated gastrointestinal malignancy, and (5) serious medical illness.

To adjust clinical characteristics, the following data were recorded for each subject: age, sex, blood type, smoking history and alcohol consumption. The study was approved by the Medical Research Committee of the Kaohsiung Veterans General Hospital. All patients and controls gave informed consent.

**Study design**

Endoscopies were performed with the Olympus GIF XV10 and GIF QX200 (Olympus Corp., Tokyo, Japan). During endoscopy, biopsies over antrum were performed for rapid urease test and histological examination. Prior to endoscopy, venous blood was drawn for serological test and myeloperoxidase genotyping. Serology was studied using a commercial IgG EIA kit (Premier H pylori; Meridian Diagnostics Inc., Cincinnati, OH). The diagnosis of H pylori infection was based on at least two positive results of histological findings, rapid urease test and serological assay.

To assess the significance of clinical characteristics, the following data were recorded for each patient: age, sex, blood type, smoking and alcohol consumption.

**Histology**

A histological examination of stomach was carried out during endoscopy for the subjects who provided informed consent for topographic histopathological study. Two specimens were taken from the antrum (pyloric gland area) and corpus (fundic gland area) at standard topographic sites. The biopsy specimens were fixed in 10% buffered formalin, embedded in paraffin, and sectioned. The sections were stained with a hematoxylin and eosin stain and a modified Giemsa stain as previously described\[^{22,23}\]. Sections were examined blinded to the patient’s clinical diagnosis. Scores of acute inflammation (neutrophil infiltration), chronic inflammation (mononuclear cell infiltration), glandular atrophy, intestinal metaplasia and H pylori density were graded from 0 to 3 as described by the updated Sydney system\[^{24}\].

**Rapid urease test**

The rapid urease test was performed according to our previous studies\[^{25}\]. Each biopsy specimen was placed immediately in 1 mL of a 10% solution of urea in deionized water (pH 6.8) to which two drops of 1% phenol red solution had been added and incubated at 37 °C for up to 24 h. If the yellowish color around the area of inserted specimen was changed to bright pink within the 24-h limit, the urease test was considered positive. In our laboratory, the sensitivity and specificity of the rapid urease test were 96% and 91%, respectively\[^{26}\].

**Myeloperoxidase genotyping**

Genomic DNA was extracted from 3 mL of whole blood by the use of a QIAamp DNA Extraction Mini Kit (QIAGEN Inc., Valencia, CA). The myeloperoxidase polymorphism analysis was performed using a PCR-restriction fragment length polymorphism method\[^{25}\]. The primers set to detect the polymorphic site at position -463 were forward primer 5'-CCGTATAAGGCAGAGAATGTTGAG-3' and reverse primer 5'-GCAAATGGTTCAAGGATTCCTTTGC-3'. The PCR product was then digested with AciI and separated on a 2% agarose gel. Individuals homozygous for the G allele had three bands at 169, 120 and 61 bp, whereas those heterozygous alleles, myeloperoxidase (G/A), had four bands at 289, 169, 120 and 61 bp. Individuals homozygous for the A allele had two bands at 61 and 289 bp.
Statistical analysis
Statistical evaluations were performed using the SPSS/Windows computer software package (Chicago, IL). Two-sample t-tests were used to compare the mean values of the variables considered continuous in the DU patients and HCs. The $\chi^2$ test with or without Yate’s correction for continuity and Fisher’s exact test when appropriate were applied to analyze the categorized variables. Differences were considered to be significant at $P<0.05$. A multivariate analysis with logistic regression method was carried out to assess the odds ratios (ORs) of the risk factors of DU. The studied variables included the following: age (<60 or ≥60 years), sex, blood type (O type or non-O type), history of smoking (<1 or ≥1 pack/wk), history of alcohol consumption (<80 or ≥80 g/d), $H\text{ pylori}$ status (presence or absence) and the carriage of myeloperoxidase allele A (yes or no).

We estimated that a 20% difference in the susceptible factor could be present in DU patients and HCs. Based on this assumption, 95 subjects had to be studied in each group to yield a statistical power of 0.80 and an $\alpha$ value of 0.05.

RESULTS
Characteristics of the patients
Table 1 shows the demographic characteristics of DU patients and controls. Patients with DU were more likely to be males and to smoke than the HCs ($P=0.012$ and 0.014, respectively). The infection rate was significantly higher in the DU group than in the control group ($P<0.001$). The two groups were similar with respect to age, blood type and history of alcohol consumption.

Table 1 Characteristics of DU patients and HCs

|          | HCs (n = 182) | DU (n = 115) | $P$  |
|----------|--------------|-------------|------|
| Age (yr) | 53.4±14.1    | 52.9±14.10  | 0.755|
| Sex      |              |             | 0.012|
| Male     | 95 (52.2)    | 77 (67.0)   |      |
| Female   | 87 (47.8)    | 38 (33.0)   |      |
| Blood group |          |             | 0.128|
| A        | 49 (26.9)    | 22 (19.1)   |      |
| B        | 55 (30.2)    | 28 (24.3)   |      |
| O        | 66 (36.3)    | 57 (49.6)   |      |
| AB       | 12 (6.6)     | 8 (7.0)     |      |
| Cigarette smokers | 37 (20.3) | 38 (33.0)   | 0.014|
| Heavy drinkers | 7 (3.8) | 8 (7.0)    | 0.233|
| $H\text{ pylori}$ infection | 87 (47.8) | 93 (80.9) | <0.001|

Myeloperoxidase genotypes in DU patients and HCs
Table 2 displays the distribution of myeloperoxidase genotypes in study groups. The distributions of this myeloperoxidase polymorphism were distinctively different between groups ($P=0.008$). The G/G, G/A and A/A genotypes were 79%, 21% and 0% respectively in HCs, and 73%, 22% and 5% respectively in DU patients. All six individuals carrying myeloperoxidase A/A genotypes were in the DU group (myeloperoxidase A/A genotype: DU, 5%; HCs, 0%; $P=0.003$).

Table 2 Genotypes and allele frequencies of myeloperoxidase gene in DU patients and HCs (n, %)

| Genotypes | HCs (n = 182) | DU (n = 115) | $P$  |
|-----------|--------------|-------------|------|
| G/G       | 143 (78.6)   | 84 (73.0)   |      |
| G/A       | 39 (21.4)    | 25 (21.7)   |      |
| A/A       | 0 (0.0)      | 6 (5.2)     |      |

Combined risk of myeloperoxidase polymorphism and $H\text{ pylori}$ infection for the development of DU
Table 3 presents the carriage rate of myeloperoxidase allele A and the $H\text{ pylori}$ status in the two studied groups. The carriage of myeloperoxidase allele A and $H\text{ pylori}$ infection were associated with an increased risk of DU with OR of 2.3 [95%CI, 0.8-8.4] and 5.8 (95%CI, 2.9-11.8), respectively. The combined risk of the carriage of myeloperoxidase allele A and $H\text{ pylori}$ infection for DU was 8.7 (95%CI, 3.5-21.8).

Comparison of histological gastritis between DU patients and HCs
Table 4 lists the histological gastritis scores in the antrum and the corpus. The scores of bacterial density, activity, inflammation, glandular atrophy and numbers of lymphoid follicles in the antrum were significantly higher in DU patients than in HCs ($P=0.010$, 0.002, 0.002, 0.001 and 0.040, respectively). The DU patients also had higher $H\text{ pylori}$ densities, activity and inflammation scores in the corpus compared with HCs ($P=0.025$, 0.021 and 0.003, respectively).

Impact of the host myeloperoxidase genotypes on $H\text{ pylori}$-related gastritis
The relationships between $H\text{ pylori}$ infection and the severity of gastritis were examined in this study. In the antrum, the activity, inflammation and atrophy scores and the number of lymphoid follicles were markedly higher in the $H\text{ pylori}$-infected individuals than in the non-infected individuals ($1.83±0.11$ vs $0.18±0.10$, $2.88±0.06$ vs $1.24±0.20$, $1.21±0.11$ vs $0.29±0.17$, and $0.57±0.12$ vs $0.00±0.00$, respectively; $P<0.001$, $P<0.001$, $P<0.001$ and $P=0.041$, respectively).

Figure 1 shows how the host myeloperoxidase genotypes impact $H\text{ pylori}$-related gastritis. Amongst the $H\text{ pylori}$-infected individuals, the myeloperoxidase allele A carriers had higher scores of $H\text{ pylori}$ densities in the antrum than the non-carriers ($2.00±0.17$ vs $1.52±0.14$, $P=0.044$). Additionally, the myeloperoxidase allele A carriers also showed a trend towards greater numbers of lymphoid follicles in the antrum and corpus than non-carriers (antrum: $0.87±0.22$ vs $0.41±0.14$, $P=0.074$; corpus: $0.20±0.11$ vs $0.04±0.04$, $P=0.089$).

DISCUSSION
The current study found that the myeloperoxidase $^*G\to A$ polymorphism was significantly associated with DU disease.
None of the 185 HCs had this special genotype. Another
in DU patients. The two study groups differed in
0%, respectively in HCs and 73%, 22% and 5%, respectively
(+), (+)  16 (8.8)                22 (19.1)                  8.7                            <0.001
(-), (+)  71 (39.0)                71 (61.7)                  5.8              <0.001
(+), (-)  23 (12.6)                   9 (7.8)                  2.3              0.1257
(-), (-)  72 (39.6)                13 (11.3) -
allele A carrier
Lymphoid follicle 0.00±0.00 0.08±0.04               0.330
Atrophy 0.00±0.00 0.23±0.07               0.133
Inflammation 0.12±0.20 1.96±0.10               0.003
Activity 0.10±0.10 0.73±0.12               0.021
Lymphoid follicle
Intestinal metaplasia 0.00±0.00 0.49±0.11               0.040
Inflammation 0.20±0.13 1.10±0.11               0.001
Activity 0.10±0.10 0.73±0.12               0.021
Lymphoid follicle 0.00±0.00 0.08±0.04               0.330

Variables including age, sex and blood group have been adjusted.

Table 4 Comparison of gastric histological findings between DU
patients and HCs

| Histological parameters | HC (n = 111) | DU (n = 115) | P       |
|-------------------------|--------------|--------------|---------|
| Antrum                  |              |              |         |
| H pylori                | 0.55±0.25    | 1.37±0.14    | 0.010   |
| Activity                | 1.60±1.07    | 2.57±0.12    | 0.002   |
| Inflammation            | 0.20±0.13    | 1.10±0.11    | 0.001   |
| Atrophy                 | 0.00±0.00    | 0.10±0.05    | 0.387   |
| Intestinal metaplasia   | 0.00±0.00    | 0.49±0.11    | 0.040   |
| Lymphoid follicle       |              |              |         |
| Corpus                  |              |              |         |
| H pylori                | 0.27±0.14    | 0.96±0.14    | 0.025   |
| Activity                | 0.10±0.10    | 0.73±0.12    | 0.021   |
| Inflammation            | 0.12±0.20    | 1.96±0.10    | 0.003   |
| Atrophy                 | 0.00±0.00    | 0.23±0.07    | 0.133   |
| Intestinal metaplasia   | 0.00±0.00    | 0.08±0.05    | 0.454   |
| Lymphoid follicle       | 0.00±0.00    | 0.08±0.04    | 0.330   |

Variables including age, sex and blood group have been adjusted.

Figure 1 Impact of host myeloperoxidase genotypes on H pylori-related gastritis.

The G/G, G/A and A/A genotypes were 79%, 21% and
0%, respectively in HCs and 73%, 22% and 5%, respectively
in DU patients. The two study groups differed in myeloperoxidase
genotype distributions. Interestingly, the six individuals
carrying myeloperoxidase A/A genotype were in the DU group.
None of the 185 HCs had this special genotype. Another
study by Roe et al[31], also showed no myeloperoxidase A/A
genotype present in 127 Korean gastritis patients. Recently,
we have examined the myeloperoxidase genotypes of 269
gastric cancer patients, and none of them had the A/A
genotypes (unpublished data). These results, taken together,
suggest that the individuals carrying myeloperoxidase A/A
 genotype are prone to develop DU.

Myeloperoxidase is an important enzyme of neutrophils,
related to oxygen burst for bacterial killing. Neutrophils
are one of the professional phagocytes in humans. They
manufacture HOCl, ·OH, peroxynitrite and many others[28].
Uniquely, myeloperoxidase readily oxidizes chloride ions to
the strong nonradical oxidant, HOCl, which have several
cytotoxic effects on bacterial cells[27-30]. Recent reports
demonstrated that myeloperoxidase activity of neutrophils
is genetically determined[19,20]. A G-to-A substitution polym-
orphism in the promoter region of myeloperoxidase gene has
been suggested to decrease gene transcription due to the
disrupted SP1 binding site[27]. Meaningless enzyme would
be available to form HOCl. In our histological study, H pylori-
infected allele A carriers had higher scores of bacterial
density. This phenomenon may be caused by low myelope-
roxidase activity in the allele A carriers, whose neutrophils
had decreased ability to generate HOCl and other reactive
oxidant species for bacterial killing[16,30].

Recent studies suggested that the bacterial load is one
of the determinants related to the outcomes of H pylori-
infected individuals. Bacterial densities of DU patients
were significantly higher than those of gastritis patients[31,32]. In
addition, the higher the H pylori load, the worse the associated
gastritis[33]. Recently, Richter-Dahlfors et al[33], demonstrated
that co-culture of antral epithelial cells with H pylori increased
basal gastrin secretion of epithelial cells. Furthermore,
Talamini et al[34], disclosed that high H pylori density was
an independent risk factor of DU. We therefore propose that
the H pylori-infected individuals with high bacterial loads
may stimulate more antral gastrin release, which can lead to
excessive acid secretion from the corpus and result in DU
diathesis.

H pylori infection is widely accepted as the most important
factor in the pathogenesis of DU and MALToma. In
our histological studies, the H pylori-infected individuals displayed
higher scores of activity, inflammation and gland atrophy
in both antrum and body than the non-infected individuals.
The *H pylori*-infected individuals who carried the *myeloperoxidase* allele A had higher bacterial scores in the antrum and a trend towards increased lymphoid follicles in the antrum and corpus than infected non-carriers (*P = 0.074 and 0.089 respectively*). Currently, the host factors affecting the growth of mucosa-associated lymphoid tissues and MALToma remain unclear. Whether the low-expression *myeloperoxidase* genotype is related to the pathogenesis of MALToma deserves further study.

The major paradox in *H pylori* research is the apparent association of the infection with divergent and mutually exclusive clinical outcomes[11,13]. The infection increases the risk of DU, a condition characterized by antral-predominant gastritis and high acid secretion while also heightening the risk of gastric cancer, a condition characterized by corpus-predominant gastritis and hypochlorhydria. Roe *et al*[31], revealed that *myeloperoxidase* genotype is a critical determinant in the pathogenesis of atrophic gastritis subsequent to *H pylori* infection. A strong positive correlation between the levels of mucosa-associated lymphoid tissues and *H pylori* infection. Aforementioned studies suggest that *myeloperoxidase* genotype may be a critical turning factor for the outcomes of *H pylori*-infected individuals.

To our knowledge, this study is the first to verify the association of *myeloperoxidase* A polymorphism with DU disease. The *H pylori*-infected allele A carriers had higher bacterial load in the antrum than did infected non-carriers. More work is mandatory to clarify the relationship between low-expression *myeloperoxidase* genotype, the reactive oxygen species of neutrophils and the fates of *H pylori*-infected individuals.

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