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Association of the Myeloperoxidase $^{-463}$G→A Polymorphism with *Helicobacter pylori*-induced Atrophic Gastritis

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Although only a minority of the infected individuals develops atrophic gastritis and the malignancy, factors governing clinical outcomes subsequent to *Helicobacter pylori (H. pylori)* infection have not yet been defined. *H. pylori* infection is characterized by extensive infiltration of neutrophils. Myeloperoxidase (MPO) in neutrophils amplifies the oxidative potential of hydrogen peroxides that induce gastric mucosal damage, thus MPO is suspected to play a role in *H. pylori*-induced gastric injury. Therefore, we explored the association of host MPO genetic polymorphism with atrophic gastritis upon *H. pylori* infection. Biopsy specimens taken from the gastric mucosa were examined histologically in 87 patients. The PCR-RFLP assay was used to characterize MPO genotypes. The distributions of MPO genotypes were MPO (G/G) 82% and MPO (G/A) 18%. None of MPO (A/A) genotype was observed. A strong positive correlation between the levels of neutrophil infiltration and gastric atrophy found only in MPO (G/G) but not in MPO (G/A) genotype. These results suggest that MPO genotype is a critical determinant in the pathogenesis of atrophic gastritis subsequent to *H. pylori* infection. Further works need to clarify the functional relevance of MPO genetic polymorphisms on gastric cell injury.

Key Words: Myeloperoxidase, Polymorphism, Helicobacter pylori, Atrophic gastritis, Host factor

INTRODUCTION

Helicobacter pylori (H. pylori) is recognized as an important cause of gastritis, and its relevance to atrophic gastritis and gastric cancer leads the WHO to classify this organism as a group I carcinogen in human (WHO, 1994). However, most *H. pylori*-infected subjects will not develop gastric cancer. It remains unclear why only a minority of the infected individuals develops atrophic gastritis and to the malignancy.

Factors governing clinical outcomes subsequent to *H. pylori* infection have not yet been defined, although variations in bacterial virulence among different strains of *H. pylori* have been suggested (Arents et al, 2000; Broutet, 2000; Leodolter et al, 2000). It is also possible that the *H. pylori* associated inflammation may interact with other causal factors such as environmental or hereditary factors related to gastric carcinogenesis. However, uncertainty exists about the contribution of hereditary factors in modulation of clinical outcomes subsequent to *H. pylori* infection.

Although *H. pylori* is known to be noninvasive, *H. pylori* infection is characterized by extensive infiltration of neutrophils. Neutrophils generate free radicals, such as superoxide anion (O_2^-) to kill bacteria. Recent study revealed that these toxic oxygen metabolites are capable of inducing apoptosis and possibly attribute to mucosal cell injury (Misso et al, 2000). Myeloperoxidase (MPO) in neutrophils amplifies the oxidative potential of hydrogen peroxides, by generating highly reactive species such as hypochlorous acid (HOCl). Moreover, ammonia generated by urease enzyme of *H. pylori* reacts with hyperchlorous acid yields cytotoxic monochloramine (NH_2Cl), a powerful oxidizing agent that can induce gastric mucosa injury (Murakami et al, 1995; Sato et al, 1999; Kodama et

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al, 2000). In addition, monochloramine is known to induce DNA fragmentation (Suzuki et al, 1997a), one of the important aspects in apoptosis, at its physiological concentration in gastric epithelial cells. These suggest the possible involvement of monochloramine formed mainly by MPO in *H. pylori* infected gastric mucosa and in gastric epithelial apoptosis (Naito et al, 1997; Suzuki et al, 1997b), and an association of MPO with gastric injury or atrophy induced by *H. pylori* infection is biologically plausible.

A G-to-A substitution polymorphism in the promoter region of the MPO gene has been suggested to decrease gene transcription (Piedrafita et al, 1996). Le Marchand et al (2000) reported the frequency of the variant A allele among Caucasian (26%), Japanese (17%) and Hawaiians (13%). Polymorphism of MPO have already been linked with numerous disease, such as acute promyelocytic leukemia (Reynolds et al, 1997), cystic fibrosis (Witko-Sarsat et al, 1996), and multiple sclerosis (Nagra et al, 1997). On the other hand, individual with low activity allele (A allele) have subsequently been found to be at decreased risk of lung cancer (Le Marchand et al, 2000; Schabath et al, 2000). However, there are no data regarding a possible link between MPO and the clinical outcomes subsequent to *H. pylori* infection. The purpose of this study was to examine the association between genetic polymorphism of the MPO gene and the development of atrophic gastritis subsequent to *H. pylori* infection.

METHODS

Subjects

Mucosal biopsy specimens were obtained from 87 patients undergoing endoscopy for upper gastrointestinal symptoms, such as epigastric pain, burning, or dyspepsia. The group consisted of 47 men and 40 women, ranging in age from 18 to 87 years (mean, 48.6 years). Patients who had taken non-steroidal anti-inflammatory drugs, proton pump inhibitors, or antibiotics during the proceeding 3 months were excluded from the study.

Histology

Formalin-fixed tissue was processed routinely in paraffin and stained with hematoxylin-eosin for light microscopy. Gastric mucosal injury was classified and scored on a scale of 0 to 3 according to the Updated Sydney System as shown in Table 1 (Dixon et al, 1996). The modified Giemsa stain was used for identification of *H. pylori* and its colonization was graded on a scale of 0 to 3. *H. pylori* infection was also confirmed or excluded in all patients by PCR and by rapid urease test (CLO-test). All histological evaluations were made without knowledge of *H. pylori* status and experimental results.

MPO genotyping

Genomic DNA was isolated from peripheral blood. PCR-RFLP assay was used to characterize the wild-type (G) and variant (A) MPO alleles at position −463. Briefly a 350 base pair region upstream of MPO gene containing the polymorphic site was amplified from PCR with primers MPOf (5'-CCGTATAGGCAGAGAATGGTGAG-3') and MPOr (5'-GCAATGGTTCAAGCGATTCTTC-3') (Le Marchand et al, 2000). Cycling conditions were: primer annealing at 56°C for

Table 1. Histologic scoring using updated sydney system (Dixon et al, 1996)

Grading the morphological variables
H. pylori density (0, 1, 2, 3)
Polymorphonuclear (PMN) cell activity (0, 1, 2, 3)
Chronic inflammation (0, 1, 2, 3)
Intestinal metaplasia (0, 1, 2, 3)
Non graded variables
Surface epithelial damage (0, 1)
Lymphoid follicle (0, 1)

Table 2. Demographic characteristics of the patients

	Male	Female	Total
N (%)	47 (54)	40 (46)	87 (100)
Age*	49.6±13.45	47.4±15.69	48.6±14.50
Hp positive (%)	68	77	72
Chronic inflam*,†	1.82±0.68	1.80±0.70	1.81±0.68
Activity*,‡	1.26±1.25	1.46±1.15	1.36±1.21
Atrophy*,§	1.5±0.62	1.39±0.64	1.45±0.63

*Values are mean±SD, †chronic inflammation (lymphocyte counts), in a scale of 0-3, ‡Activity (neutrophil counts), in a scale of 0-3, §atrophy, in a scale of 0-3 according to the Updated Sydney System.

1 min, polymerization at 72°C for 1 min and denaturation at 94°C for 1 min. Thirty cycles were carried out. A final polymerization step of 72°C for 5 min was carried out to complete the elongation processes. The PCR product (10 μl) was then digested with *Aci*I and separated on a 2% agarose gel containing 0.5 μg/ml ethidium bromide.

Statistical analysis

Generalized linear regression model (PC-SAS, V 6.12) was used to test the association of MPO genotypes with atrophic gastritis induced by *H. pylori* infection.

RESULTS

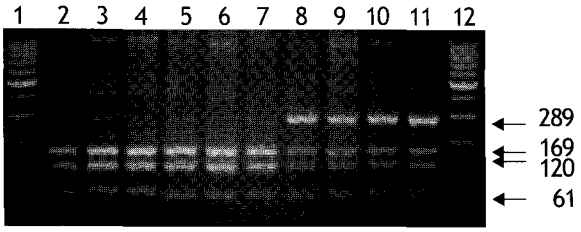
Basic characteristics

The study included 87 patients with various degrees of gastritis. Table 2 shows the basic characteristics of the patients according to age and the degree of gastric mucosal injury based on the Updated Sydney System (Dixon et al, 1996). Patients infected by *H. pylori* were defined by positive results in all of the methods; PCR, rapid urase test, and identification of the organism on histology using the antral biopsy specimens. *H. pylori* infection was detected in 72% of the patients studied. There was no age or gender difference in the correlation between the degrees of *H. pylori* colonization, neutrophil infiltration and gastric atrophy.

MPO genotyping

A G→A substitution at position −463 in the promoter region of the MPO gene leads to the loss of an additional *Aci*I restriction site, which was used to distinguish the two allele (Fig. 1A). An invariant

(A)



(B)

MPO Genotype	G/G	G/A	A/A
N (%)	70 (82)	17 (18)	0 (0)

Fig. 1. (A) Electrophoresis on a 2% agarose gel of the AciI-digested PCR-amplified 350 bp fragment from MPO promoter region containing the polymorphic site. The $^{-463}$G→A substitution present in variant (A) MPO allele leads to the loss of a AciI site within 350 bp amplification fragment that was used to distinguish the two alleles. Individuals homozygous for the G allele have three bands at 169, 120, and 61 bp (lines 2~7), whereas those heterozygous allele, MPO (G/A), have four bands at 289, 169, 120, and 61 bp (lines 8~11). Individual homozygous for the A allele was not found in 87 samples tested. The size of fragments was estimated using the 100 bp ladder (line 1 and 12). (B) The distribution of MPO genotypes.

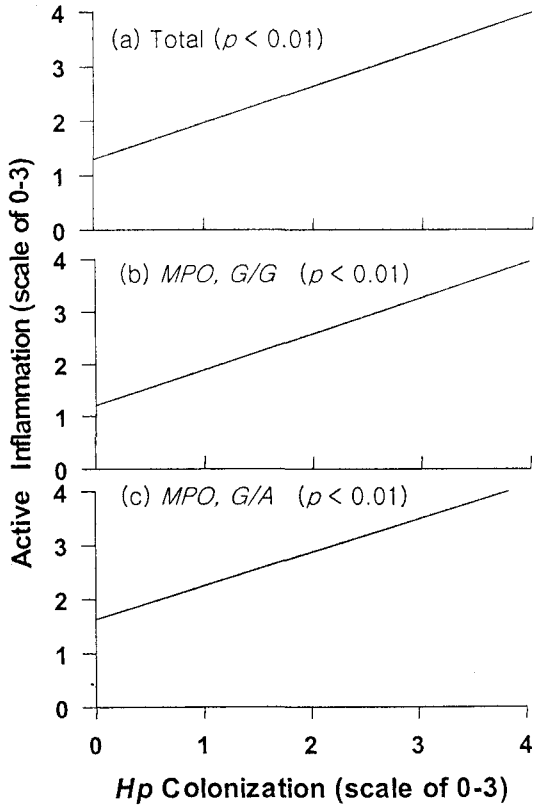


Fig. 2. The correlation between the degree of *Hp* colonization and active inflammation according to the MPO polymorphism (a) Total (n=87); y=0.6718x+1.289, (b) *MPO (G/G);* y=0.6854x+1.204, (c) *MPO (G/A);* y=0.6184x+0.6208.

*Aci*I site present in both alleles yields a 61-bp fragment that serves as an internal control. The distributions of MPO genotypes were MPO (G/G) 82% and MPO (G/A) 18%. None of MPO (A/A) allele was observed in 87 samples studied (Fig. 1B).

The degrees of H. pylori colonization and neutrophil infiltration

Fig. 2 shows the relationship between the degrees of *H. pylori* colonization and neutrophil counts as a marker of active inflammation. Generalized linear regression analysis showed significant positive correlations between *H. pylori* load and the extent of neutrophil infiltration. MPO genotypes were unrelated to degree of neutrophil infiltration in gastric mucosa as a marker of active inflammation upon *H. pylori* infection (MPO (G/G): r=0.6854, $p<0.01$; MPO (G/A): r=0.6184, $p<0.01$); Total: r=0.6718, $p<0.01$).

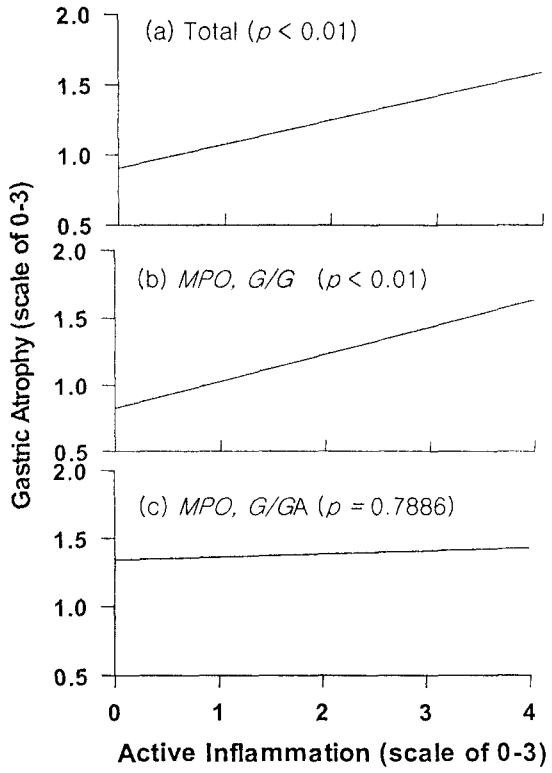


Fig. 3. The correlation between the degree of active inflammation and according to the MPO polymorphism (a) Total (n=87); y=0.1712x+0.9051, (b) *MPO, G/G* (n=80); y=0.2014x+0.0.8241, (c) *MPO, G/A* (n=17); y=0.0216x+1.0121.

The degrees of active inflammation and gastric atrophy

Generalized linear regression analysis showed significantly positive correlations between neutrophil counts as a marker of active inflammation and the degree of gastric atrophy (Total: r=0.1712, $p<0.01$; MPO (G/G): r=0.2014, $p<0.01$) (Fig. 3A & 3B). However, no correlation was recognized between the two parameters in patients with MPO (G/A) genotypes (r=0.0216, p=0.7886) (Fig. 3C). No suggestion could be made on MPO (A/A) genotype, since it was not found in 87 samples studied.

DISCUSSION

We studied the association of MPO genetic polymorphism with the development of gastric atrophy subsequent to *H. pylori* infection. Although the association of *H. pylori* infection with a higher risk of gastric carcinoma has been reported repeatedly by epidemiological studies, the precise mechanisms for these phenomena are unclear. It is generally accepted that *H. pylori* is involved in gastric carcinogenesis as proposed by the Correa's model of the development of gastric carcinoma from normal gastric tissue through superficial gastritis, multifocal atrophic gastritis, intestinal metaplasia and dysplasia to carcinoma (Konturek et al, 1999). Nevertheless, it remains obscure why only a minority of those individuals infected develops the malignancy, even in area with high prevalence of both *H. pylori* infection and gastric carcinoma.

Diversity of virulence determinants of *H. pylori* strains, such as *vacA, cagA* PAI, *iceA*, *babA* and Lewis antigen, have been extensively investigated as pertinent determinants of *H. pylori*-related disease manifestations associated with this chronic infection (Arents et al, 2000; Broutet, 2000; Leodolter et al, 2000). Despite of extensive studies on the role of the status of virulence factor gene as determinant of clinical outcomes, it remains unanswered yet.

Conversely, host response against *H. pylori* could be also another important factor forming disease diversity. El-Omar et al (2000) reported that interleukin-1 gene cluster polymorphisms suspected of enhancing production of interleukin-1-beta are associated with an increased risk of both hypochlorhydria induced by *H. pylori* and gastric cancer. Depletion of

endogenous gastric GSH stores in chronic *H. pylori* infection was suggested to contribute to mutagenesis and other deleterious consequence of oxidative stress (Shirin et al, 2000).

Recently Mizuki et al (2000) reported that there was a significant positive correlation between the number of epithelial apoptotic cell and oxidative burst in neutrophils. Increased neutrophil oxidative burst stimulated by *H. pylori* then enhanced gastric mucosal DNA damage and consequent atrophic gastritis and gastric cancer (Abe et al, 2000; Leakey et al, 2000). Neutrophils utilize the H_2O_2-myeloperoxidase (MPO)-halide system to generate a powerful oxidant, Hypochlorous acid (HOCl). Since neutrophils invade an area infected by *H. pylori*, neutrphils derived hypochlorous acid reacts with ammonia produced by *H. pylori* to yield cytotoxic monochloramine (NH_2Cl), a powerful oxidizing agent capable of destroying cellular components (Murakami et al, 1995; Sato et al, 1999).

Our results showed a strong positive correlation between the level of neutrophil infiltration as a marker of active inflammation and *H. pylori* colonization in gastric mucosa. Moreover, the degree of atrophic gastritis was significantly related to the level of neutrophils infiltration. These findings support the previous reports (Abe et al, 2000; Leakey et al, 2000; Yoshikawa & Naito, 2000), thus enhancement of neutrophils infiltration due to the *H. pylori* infection should be taken into account when evaluating the relationship between *H. pylori* infection and development of gastric atrophy.

An association of MPO with gastric injury upon *H. pylori* infection is biologically plausible. MPO and reactive by-products have been linked to oxidative stress (Podrez, 2000), DNA-strand breakage (Suzuki et al, 1998) and bioactivation of carcinogen (William, 2001). Van Rensburg (1992) reported that Interactions between the neutrophil-derived reactive oxidants H_2O_2 and HOCl are probably involved in the etiology of inflammation-related cancer. The $^{-463}G \rightarrow A$ substitution in the promoter region of the MPO gene has been associated with a decreased transcriptional activity due to the disruption of SP1 binding site (Piedrafita et al, 1996), thus less enzyme would ultimately be available for formation of hypochlorous acid and monochloramine. Moreover, it is reasonable to assume that humans have a variable capacity to activate carcinogens when the allelic variant relevant for the phenotype exists. Given our results, the strong positive correlation between the levels of neutrophil infiltration and gastric atrophy found only in MPO (G/G) but not in MPO (G/A) genotype, possible association of MPO genotype and *H pylori*-induced gastric atrophy might be an attractive hypothesis (Fig. 4). Unfortunately, we could not find MPO (A/A) genotype in 87 samples studied. Published reports on prevalence of MPO (A/A) genotype was approximately 5% in Caucasian (Kantarci et al, 2000; Le Marchand, 2000; Misra et al, 2001) and 3% in Japanese (Le Marchand, 2000). The absence of MPO (A/A) genotype shown in our study reflects ethnic differences in MPO genetic polymorphism.

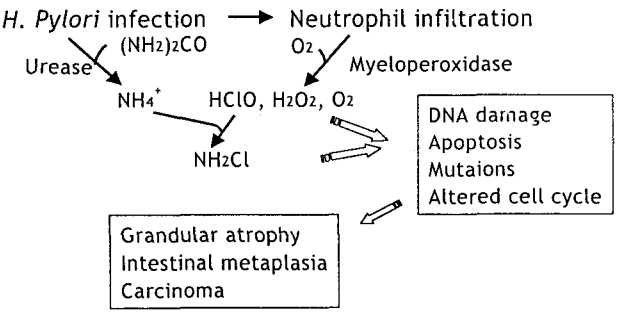


Fig. 4. Hypothesis in association of MPO and *H. pylori* with development of atrophic gastritis, intestinal metaplasia and carcinoma.

In the present study, we found a strong positive correlation between the degrees of gastric atrophy and neutrophil infiltration on the level of *H. pylori* infection in MPO (G/G) genotype. However, enhanced neutrophil infiltration did not associated with the degree of gastric atrophy among MPO variant individuals. These results suggest that MPO genotype is a critical determinant in the pathogenesis of atrophic gastritis induced by *H. pylori* infection. To our knowledge, this is the first study to examine the relationship between MPO genotype and *H. pylori* induced atrophic gastritis. However, further works need to clarify the functional relevance of MPO genetic polymorphisms on gastric cell injury and to confirm the role of MPO (A/A) genotype on gastric mucosal damage or atrophy and to gastric cancer induced by *H. pylori* infection in large population studies.

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