

Functional polymorphisms in the promoter region of macrophage migration inhibitory factor and chronic gastritis

TOMIYASU ARISAWA, TOMOMITSU TAHARA, TOMOYUKI SHIBATA, MITSUO NAGASAKA, MASAKATSU NAKAMURA, YOSHIO KAMIYA, HIROSHI FUJITA, MASAHICO NAKAMURA, DAISUKE YOSHIOKA, YUKO ARIMA, MASAOKI OKUBO, ICHIRO HIRATA and HIROSHI NAKANO

Department of Gastroenterology, Fujita Health University School of Medicine,
1-98 Dengakugakubo, Kutsukake-cho, Toyoake 470-1192, Japan

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Abstract. Macrophage migration inhibitory factor (MIF) is a key proinflammatory mediator, which plays a pivotal role in inflammatory and immune diseases. We attempted to clarify associations of the functional polymorphisms of the MIF gene promoter with the development of chronic gastritis. The study was performed with 290 stocked DNAs from subjects with no evidence of gastric malignancy. We employed the PCR-SSCP method to detect gene polymorphisms. The severity of histological chronic gastritis in antral biopsy specimens was classified according to the updated Sydney system. Both the 7/7-CATT repeat at position -794 and the -173 C/C genotypes were significantly associated with a risk of developing severe gastric mucosal atrophy (OR, 9.69; 95% CI, 1.29-72.5; and OR, 4.60; 95% CI, 1.05-20.2, respectively). In subjects younger than 60 years old, the number of 7-CATT alleles was significantly correlated with both the activity and inflammation scores ($p=0.0079$ and 0.0080 , respectively). Our results suggested that functional promoter polymorphisms of the MIF gene might be associated with the severity of gastric mucosal inflammation in younger subjects and with the subsequent development of mucosal atrophy.

Introduction

Macrophage migration inhibitory factor (MIF) is a key proinflammatory mediator, which plays a pivotal role in inflammatory and immune diseases (1-5). It contributes toward an excessive inflammatory response both directly via an induction of proinflammatory cytokine secretion (6) and indirectly through its ability to override the anti-inflammatory activity of glucocorticoids (7). MIF has been shown to

contribute toward an exaggerated gram-negative response through its ability to induce Toll-like receptor 4 (TLR4), a key receptor responsible for LPS-induced inflammatory cytokine production (8). It is well known that *Helicobacter pylori* (*H. pylori*), one of the gram-negative bacterium, colonizes in the human stomach and causes gastric inflammation and peptic ulcer disease. Recently, Xia *et al* reported that *H. pylori* infection increased MIF expression in both gastric inflammatory and epithelial cells (9), thus MIF may play an important role in *H. pylori*-related gastric inflammation.

Polymorphisms with potential functional relevance have been identified in the MIF gene promoter; an SNP at position -173 (G to C) (10) and a tetranucleotide CATT repeat beginning at nucleotide position -794 (11) have been found to be associated with altered levels of MIF gene transcription *in vitro*. Although it has been demonstrated that the functional importance of these variants includes a of significant association with several immune-mediated inflammatory diseases (10-12), the role of these polymorphisms in the development of chronic gastritis remains unclear.

In the present study, we attempted to clarify associations of G-173C and -794 CATT repeats in the MIF gene promoter with the development of chronic gastritis.

Materials and methods

Clinical samples. We randomly selected 300 samples from our stocked DNA from patients who were enrolled at the Endoscopy Center of Fujita Health University Hospital in 2006. All these patients underwent upper gastrointestinal endoscopy, and biopsy specimens were taken from antral mucosa. Part of each specimen was fixed in 10% buffered formalin and embedded in paraffin, while the other part was immediately frozen and stored at -80°C . Finally, the study population comprised 290 subjects with no neoplastic lesions whose DNA was clearly analyzed.

All histological diagnoses were made at the Department of Pathology of our hospital. The severity of chronic gastritis was also classified according to the updated Sydney system (13) by a pathologist who had no access to any clinical information. According to the severity of gastric mucosal atrophy, the subjects were divided into two groups as

Correspondence to: Dr Tomiyasu Arisawa, Department of Gastroenterology, Fujita Health University School of Medicine, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake 470-1192, Japan
E-mail: tarisawa@fujita-hu.ac.jp

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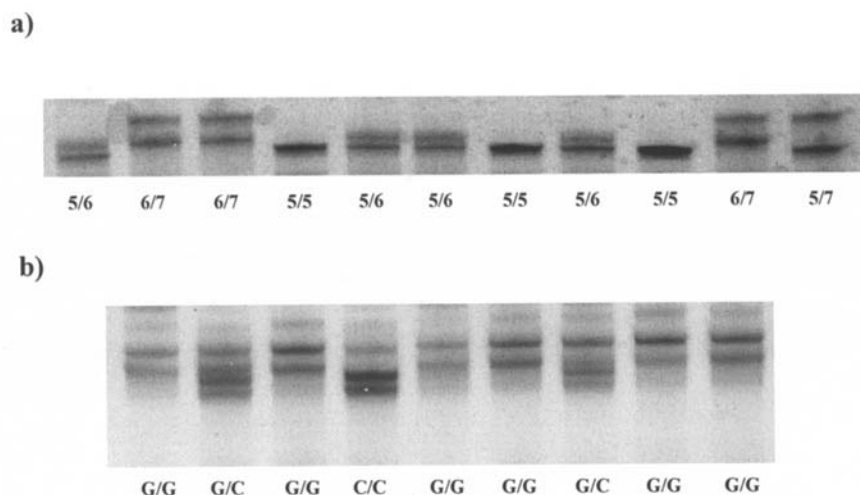


Figure 1. The images of PCR-SSCP using clinical samples. (a) Identification to a tetranucleotide (CATT) repeat (5-7) at position -794. (b) Identification to a base (G/C) at position -173.

follows: the severe atrophy (SA) group (atrophy score ≥ 2 or metaplasia score ≥ 2) and the non-atrophy group (the remaining subjects). *H. pylori* infection status was assessed by serology, histological examination, or the urea breath test. Patients were diagnosed as having infection when at least one of the diagnostic tests was positive.

The Ethics Committee of Fujita Health University School of Medicine approved the protocol, and written informed consent was obtained from all of the participating subjects.

Genotyping of polymorphisms. Sample stocked DNAs isolated from biopsy specimens or peripheral blood were used. Polymorphism was genotyped by the PCR-SSCP method as reported previously (14,15). To detect -794 CATT repeats, using the primer pair (MIFTR forward: 5'-TGATCC AGTTGCTGCCTTGTC-3', and MIFTR reverse: 5'-TCCA CTAATGGTAAACTCGGGGAC-3'), PCR was carried out in a volume of 20 μ l containing 0.1 μ g of genomic DNA. The DNA was denatured at 95°C for 3 min, followed by 35 cycles at 95°C for 30 sec, 62°C for 40 sec, and 72°C for 45 sec, with final extension at 72°C for 5 min. Thereafter, 2 μ l of the PCR product was denatured with 10 μ l of formamide (Sigma-Aldrich Co., St. Louis, USA) at 90°C for 5 min. SSCP was carried out at 6°C using a GenePhor DNA separation system with GeneGel Excel 12.5/24 (Amersham Biosciences Corp., USA), after which the denatured single-strand DNA bands were detected using a DNA Silver Staining kit (Amersham Biosciences Corp.).

To detect the G-173C polymorphism, using the primer set (MIF173 forward: 5'-TCTAGCCGCCAAGTGGAGAACA-3' and MIF173 reverse: 5'-ACTGTGGTCCCGCCTTTG TGA-3'), the PCR reaction was carried out at a 60°C annealing temperature as described above. SSCP was also carried out as described above.

Statistical analysis. The odds ratio (OR) and 95% confidence intervals (CI) were estimated by logistic regression analysis after adjustment for age, gender and *H. pylori* infection status. The Mann-Whitney U test and linear regression analysis were employed to assess the association between MIF

polymorphism and the updated Sydney system scores. For all analyses, the level of significance was set at $p < 0.05$.

Results

The characteristics of subjects and the frequencies of genotypes. As shown in Fig. 1, single-strand DNAs of both -794 repeats and G-173C were clearly separated by SSCP. A single-strand band of the 8-CATT repeat was not detected in any of the 290 subjects. These polymorphisms were in significant linkage disequilibrium, with the -173C allele strongly associated with the 7-CATT repeat allele. The most frequent haplotypes were G-5-CATT, G-6-CATT and C-7-CATT, which constituted about 90% of the haplotypes.

With respect to gastric mucosal atrophy, 103 of the subjects were classified into the SA group (Table I). The male/female ratio was higher in the SA group than in the non-atrophy group. Regarding the *H. pylori*-positive rate, there was a significant difference between the 2 groups (SA > non-atrophy). The frequencies of n-CATT and -173C alleles were not significantly different between the 2 groups.

The association between the -794 CATT repeats and gastric mucosal atrophy. Overall, infection with *H. pylori* was strongly associated with the development of gastric mucosal atrophy. By unadjusted analysis, both male gender and the 7/7-CATT genotype were significantly associated with gastric mucosal atrophy, and the 7/7-CATT genotype was also significantly associated after adjustment for age, gender and *H. pylori* infection status (OR, 9.69; 95% CI, 1.29-72.5; Table II). The frequency of the 5/5-CATT genotype was not associated with the gastric mucosal atrophy. In addition, a significant association between the gastric mucosal atrophy and the -173C/C genotype was found by both unadjusted and adjusted analysis (OR, 2.77; 95% CI, 1.02-7.50; and OR, 4.60; 95% CI, 1.05-20.2, respectively; Table III).

The relationship between MIF genotypes and the infiltration of inflammatory cells into gastric mucosa. As shown in Fig. 2, there was no significant relationship between MIF gene

	Total	Non-atrophy	Severe atrophy
No. of subjects	290	187	103
Mean age \pm SD	60.9 \pm 13.0	59.6 \pm 14.1	63.4 \pm 10.5
Male:female	178:112	101:86	77:26
<i>H. pylori</i> -positive rate	66.2%	48.1%	99.0%
n-CATT repeat			
5/5	46	30	16
5/6	87	53	34
5/7	37	25	12
6/6	55	37	18
6/7	55	39	16
7/7	10	3	7
5-CATT frequency	37.2%	36.9%	37.9%
6-CATT frequency	43.4%	44.4%	41.7%
7-CATT frequency	19.3%	18.7%	20.4%
G-173C genotype			
G/G	170	106	64
G/C	103	74	29
C/C	17	7	10
-173C frequency	23.6%	23.5%	23.8%

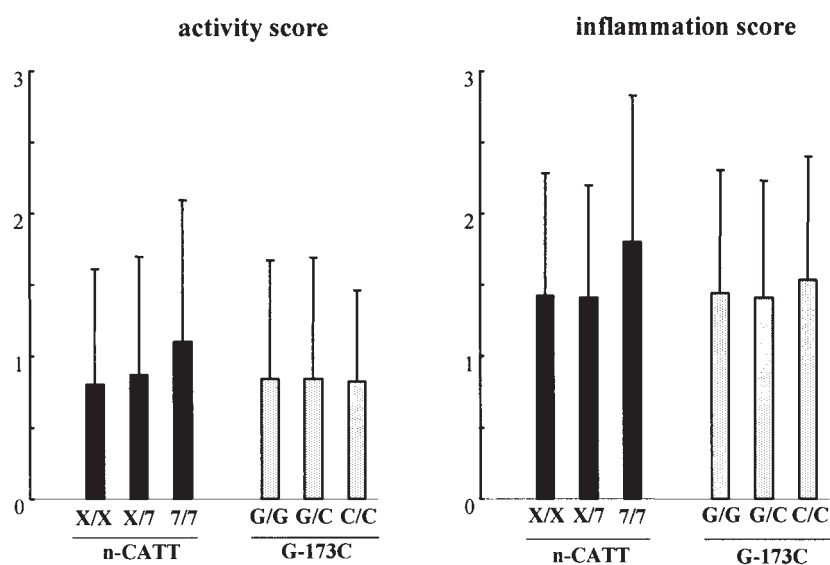


Figure 2. The association between promoter polymorphisms of the MIF gene and the inflammation or activity score. There were no significant differences among genotypes. X, 5- or 6-CATT.

promoter genotypes and the activity or inflammation score. However, we recognized the significant interaction between age and the number of 7-CATT alleles with the increase of activity and inflammation scores by ANOVA ($p=0.0067$ and $p=0.0078$, respectively), so we investigated the association of MIF genotypes with the activity and inflammation scores in subjects younger than 60 years old. As shown in Fig. 3, significant relationships between the number of 7-CATT alleles and both the activity and inflammation scores were seen by linear regression analysis ($p=0.0079$, $r=0.24$; and $p=0.0080$, $r=0.24$, respectively).

Discussion

In the present study, we found a significant association between gastric mucosal atrophy and two promoter polymorphisms of the MIF gene. The -173C/C and 7/7-CATT genotypes were significantly associated with an increased risk for the development of atrophic gastritis. Since -173C was strongly associated with 7-CATT repeats, the C-7-CATT haplotype may be associated with gastric mucosal atrophy.

Infection with *H. pylori* first induces chronic superficial gastritis, which can progress to chronic atrophic gastritis,

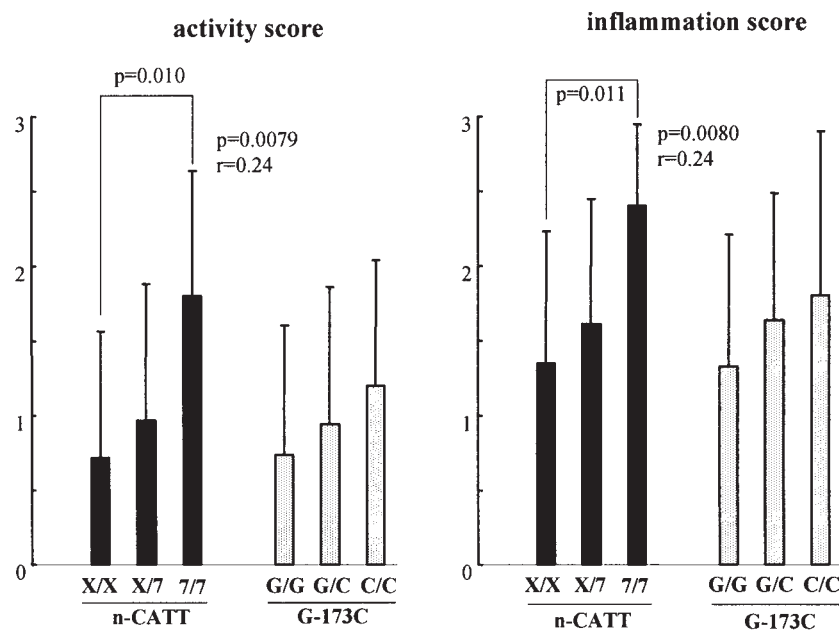


Figure 3. The association between promoter polymorphisms of the MIF gene and the inflammation or activity score in subjects younger than 60 years old. The comparison among two groups was performed by the Mann-Whitney U test, and the associations between the number of 7-CATT alleles and each score were assessed by linear regression analysis. X, 5- or 6-CATT.

Table II. Association between gastric mucosal atrophy and various risk factors.

	OR (95% confidence intervals)	
	Unadjusted odds ratio	Adjusted odds ratio ^a
5/5-CATT	0.96 (0.50-1.86)	1.09 (0.48-2.48)
7/7-CATT	4.47 (1.13-17.7)	9.69 (1.29-72.5)
Male gender	2.52 (1.49-4.28)	1.89 (0.99-3.59)
Elder age	1.02 (1.00-1.05)	1.04 (1.02-1.07)
<i>H. pylori</i> infected	110 (15.0-804)	118 (15.8-888)

^aAdjusted for gender, age, and *H. pylori* infection status.

Table III. Association between gastric mucosal atrophy and various risk factors.

	OR (95% confidence intervals)	
	Unadjusted odds ratio	Adjusted odds ratio ^a
-173 C/C	2.77 (1.02-7.50)	4.60 (1.05-20.2)
Male gender	2.52 (1.49-4.28)	1.72 (0.91-3.23)
Elder age	1.02 (1.00-1.05)	1.04 (1.01-1.07)
<i>H. pylori</i> infected	110 (15.0-804)	125 (16.6-951)

^aAdjusted for gender, age, and *H. pylori* infection status.

intestinal metaplasia, and dysplasia that leads toward gastric carcinoma (16). However, there are marked interindividual differences in the extent of inflammation among persons with *H. pylori* infection, so clinical consequences only develop in a small subgroup. To determine the important genetic factors, several studies concerning the association between gene polymorphisms and gastric inflammation during *H. pylori* infection were performed (14,15,17,18). MIF was originally identified as an activity isolated from T lymphocytes that was capable of inhibiting the random migration of macrophages (19,20). The human MIF cDNA was finally cloned in 1989 (21). Many studies have shown MIF to be a key modulator of many chronic and disabling human disorders, such as rheumatoid arthritis (22), sepsis (23), acute respiratory syndrome (24), and atopic diseases (25,26). An important role of MIF in gastric disorders has also been shown, such as gastric inflammation (9), ulcer (27) and carcinogenesis (28). It is

well known that a polarized T helper 1 immune response occurs in *H. pylori* infection (29). MIF directly activates or promotes cytokine expression [TNF (30), IL-2 (31), IL-8 (32) and INF- γ (33)]. Furthermore, Xia *et al* have reported that *H. pylori* stimulates MIF release in monocytes and expression of MIF in gastric mucosa (9). However, there is no report regarding the association between MIF gene promoter functional polymorphisms and gastric disorders, although several studies have shown significant associations of these polymorphisms with cystic fibrosis (34), psoriasis (35), and atopic disorders (36). Therefore, we hypothesized that MIF functional polymorphisms might be associated with the development of chronic gastritis.

Promoter sequence analysis indicated that the -173C allele creates a potential activator protein 4 transcription factor binding site (10), and levels of MIF expression significantly differed among G-173C genotypes in a cell type manner. Regarding CATT repeats, the 5-CATT allele was shown to



associated with lower basal and stimulated MIF promoter *vitro* than 6-, 7- and 8-CATT alleles (11). Donn *et al* showed that increasing CATT repeats with the -173C allele significantly increased the promoter activity in a T lymphoblast cell line (10). Thus, the -173C allele and 7-CATT seemed to promote the production of MIF, although there is no clear relationship between these polymorphisms and the transcriptional regulation of the MIF gene.

Results from our study suggested that both the 7/7-CATT repeat and the -173 C/C genotypes, as well as *H. pylori* infection and elder age, were significantly associated with the development of gastric mucosal atrophy. Baugh *et al* reported the correlation of the 5/5-CATT repeat with low disease severity in rheumatoid arthritis patients (12), and Hizawa *et al* also reported an increased risk of non-5-CATT carriers for atopy (36). Donn *et al* demonstrated that the -173C/7-CATT haplotype is of importance in the susceptibility to psoriasis (35). Thus, there is no wonder that both the 7-CATT repeats and the -173C allele are significantly associated with the development of gastric mucosal atrophy. However, a significant relationship between the number of 7-CATT alleles and inflammatory cell infiltrations was seen only in subjects younger than 60 years old. It has been shown that long-term exposure to *H. pylori* is a significant risk factor for the progression of atrophic and metaplastic gastritis (37,38). In elder subjects, gastric mucosal inflammation may subside because of the progression of gastric atrophy and metaplasia. In addition, a lower serum MIF level was exhibited in elder compared to younger rats (39). Therefore, MIF may promote the gastric mucosal inflammation in younger subjects, whereas it may not in elder subjects.

In conclusion, functional polymorphisms of the MIF gene promoter may be associated with the severity of gastric mucosal inflammation in younger subjects and with the subsequent development of mucosal atrophy.

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