Genetic polymorphisms of molecules associated with inflammation and immune response in Japanese subjects with functional dyspepsia

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

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

Received June 18, 2007; Accepted July 30, 2007

Abstract. Inflammatory changes in the gastric mucosa are commonly observed in Japanese patients with functional dyspepsia (FD). However, detailed data regarding the relationship between the genetic regulatory factors of inflammation and FD are not available. We investigated the associations between FD and genetic polymorphisms of molecules associated with inflammation or immune response (IL-17A, -17F and MIF). The study was performed with 278 subjects (188 with no upper abdominal symptoms and 90 with FD according to the Roma III criteria). We employed the PCR-SSCP (multiplex PCR for IL-17A and -17F) method to detect the gene polymorphisms. Overall, the polymorphisms of the IL-17A, -17F and MIF genes were not correlated with the susceptibility to FD. However, the MIF -173C allele carrier had a significantly increased risk for the development of epigastric pain syndrome (EPS) of FD (OR, 2.12; 95% CI, 1.00-4.49; p=0.0497). In *Helicobacter pylori* (*H. pylori*)-infected cases, the number of IL-17F 7488T alleles was positively correlated with the development of EPS (OR, 11.3; 95% CI, 1.23-103.2; p=0.032), while the IL-17F T/T homozygote and the MIF -173C carrier had an increased risk for EPS (OR, 10.4; 95% CI, 1.17-92.3; p=0.036 and OR, 3.66; 95% CI, 1.19-11.3; p=0.024, respectively). In addition, a significant interaction between the IL-17F 7488 polymorphism and *H. pylori* infection was shown to increase the activity and inflammation scores (p=0.043 and 0.042, respectively). There were no significant associations between the IL-17A polymorphism and FD. Our results provide the first evidence that the IL-17F and MIF gene polymorphisms are significantly associated with the development of FD, particularly EPS, a subgroup of FD, in *H. pylori*-infected subjects. The genetic polymorphisms of inflammation or immune response-related molecules are involved in the development of one of the FD subgroups via *H. pylori*-induced gastric inflammation.

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

Key words: interleukin-17, macrophage migration inhibitory factor, genetic polymorphism, functional dyspepsia, *Helicobacter pylori*

Introduction

Functional dyspepsia (FD) is a common clinical syndrome characterized by the presence of recurrent or chronic upper abdominal symptoms, such as epigastric pain, early satiety, and fullness, without anatomical or biochemical abnormalities identifiable by conventional diagnostic tests, including upper gastrointestinal endoscopy (1). Talley *et al* have shown that up to 25% of the population experience these symptoms (2). FD is a heterogeneous condition indicated by a variety of different pathophysiologic mechanisms which have been demonstrated in this disorder (3), thus, FD does not have a well-established pathophysiology. Gastrointestinal motor abnormalities (4), altered visceral sensation (5) and psychosocial factors (6) have been reported to play essential roles in the pathophysiology of FD. Locke *et al* reported familial clustering of FD (7). In addition, it was reported that the G-protein ß3 subunit gene polymorphism is associated with FD (8,9). These facts suggest that genetic factors may play a significant role in the development of FD.

On the other hand, *Helicobacter pylori* (*H. pylori*) infection is a powerful pathogenic factor, and many studies have revealed a strong association between infection of this organism and gastric disorders. *H. pylori* infection usually leads to persistent colonization and chronic gastric inflammation. According to the Roma III criteria (10), *H. pylori*-infected patients, who had some chronic or recurrent upper abdominal symptoms with neither ulceration nor erosion in the gastroduodenal mucosa by gastrointestinal endoscopy, were diagnosed with FD. That is, there is a possibility that one of the FD subgroups is associated with gastric mucosal inflammation, although adult FD patients frequently have motility abnormalities of the stomach and the upper small

bowel including antral hypomotility and delayed gastric emptying (11-13).

Interleukin-17 (IL-17) is a relatively newly described cytokine which bridges the adaptive and innate immune systems. IL-17 family members belong to a distinct category of cytokines and play a role in coordinating local tissue inflammation by inducing the release of proinflammatory and neutrophil-mobilizing cytokines (14). In addition, IL-17A and -17F share similar functions in terms of their ability to induce chemokines that are important in neutrophil recruitment and activation. Recently, Kawaguchi *et al* reported that the IL-17F 7488T/C (rs763780), which causes a His-to-Arg substitution at amino acid 161 (H161R) variant, influences the risk of asthma and is a natural IL-17F antagonist in the known polymorphisms of the IL-17 gene (15). Macrophage migration inhibitory factor (MIF) was originally identified as an activity isolated from T lymphocytes which was capable of inhibiting the random migration of macrophages (16). MIF contributes toward an excessive inflammatory response both directly and via an induction of proinflammatory cytokine secretion (17). Polymorphisms with potential functional relevance have been identified in the MIF gene promoter; a SNP at position -173 (G to C) (18) and a tetranucleotide CATT repeat beginning at nucleotide position -794 (19) were found to be associated with altered levels of MIF gene transcription *in vitro*. Important roles of both IL-17 and MIF in the inflammatory response to *H. pylori* infection have been demonstrated (20,21). Therefore, we hypothesized the possibility that the functional polymorphisms of IL-17 and MIF genes may be associated with the development and the pathophysiological features of FD.

In the present study, we investigated the association between the polymorphisms of IL-17A (G-197A, rs2275913), -17F (7488T/C, rs763780), and MIF (G-173C and -794 tetranucleotide repeats) and FD diagnosed according to the Roma III criteria.

Materials and methods

Clinical samples. We randomly selected 300 samples from our stocked DNA obtained in 2006. Two hundred subjects, enrolled at the Endoscopy Center of Fujita Health University Hospital, had previously undergone upper gastrointestinal endoscopy to check for gastric cancer followed by a barium X-ray examination as a health check or for the complaint of abdominal discomfort. One hundred subjects were healthy volunteers with no abdominal symptoms. For certain subjects, the severity of gastric inflammation was assessed according to the updated Sydney system using biopsy specimens taken from the antral mucosa by a pathologist who had no access to any clinical information. Subjects who had significant upper gastrointestinal findings such as peptic ulcer diseases, reflex esophagitis and malignancies were excluded from this study. Patients with severe systemic diseases and malignancies in other organs, or who had received non-steroidal anti-inflammatory drugs, antibiotics and *H. pylori* eradication therapy were also excluded.

According to the Roma III criteria, 90 dyspeptic patients were identified as having a primary complaint of either continuous or intermittent dyspepsia for 3 months, onset at least 6 months before, predominantly located in the upper abdomen. In 90 dyspeptic patients, 46 and 39 patients were diagnosed with epigastric pain syndrome (EPS) and post-prandial syndrome (PDS), respectively. Subjects who had no dyspeptic symptoms within the last 12 months were considered as non-dyspepsia subjects. Finally, the study population comprised 278 subjects whose DNA was clearly analyzed, including 94 healthy volunteers.

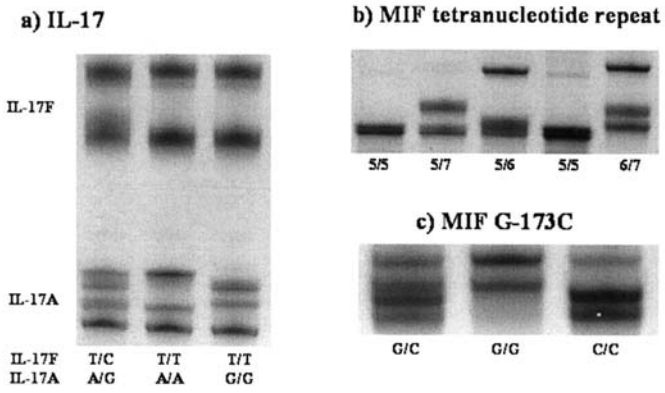

Figure 1. The images of multiplex PCR-SSCP using clinical samples. (a) IL-17A and -17F, (b) MIF tetranucleotide repeat and (c) MIF G-173C single-strand DNAs were clearly separated by SSCP.

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

Detection of H. pylori infection. *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.

Genotyping of polymorphisms. Polymorphism was genotyped by PCR-SSCP method as reported previously (22,23). To detect the IL-17A and -17F polymorphisms, using the primer pairs (IL-17AF, 5'-aacaagtaagaatgaaaagaggacatggt-3'; IL-17AR, 5'-cccccaatgaggtcatagaagaatc-3'; IL-17FF, 5'-gtgtag gaacttgggctgcatcaat-3'; and IL-17FR: 5'-agtggatatgcacctcttac tgcaca-3', respectively), one-tube multiplex PCR was carried out in a volume of 20 μl containing 0.1 μg of genomic DNA. The DNA was denatured at 96˚C for 90 sec, followed by 35 cycles at 96˚C for 15 sec, 58˚C for 30 sec, and 72˚C for 45 sec, with a final extension at 72˚C for 3 min. Thereafter, 2 μl of the PCR product was denatured with 10 μl of formamide (Sigma-Aldrich Co., St. Louis, USA) at 90˚C for 5 min. SSCP was carried out at 6 or 12˚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 MIF -794 CATT repeats and the G-173C polymorphism, using the primer pairs (MIFTRF, 5'-tgatcca gttgctgccttgtc-3'; MIFTRR, 5'-tccactaatggtaaactcggggac-3'; MIF173F, 5'-tctagccgccaagtggagaaca-3'; and MIF173R, 5'-act gtggtcccgccttttgtga-3', respectively), PCR was carried out in a volume of 20 μl containing 0.1 μg of genomic DNA. The

Table I. Characteristics of the subjects and frequency of genotypes.

	Non-symptom	FD	EPS	PDS
Number of subjects	188	90	46	39
Mean age ± SD	43.6±19.7	60.2±13.1	58.2±14.3	62.2±10.9
Male:female	114:74	34:56	16:30	15:24
H. pylori infected	60.6%	54.4%	45.7%	48.7%
IL-17A (rs2275913)				
G/G	59	32	18	14
G/A	110	51	24	23
A/A	19	6	4	1
A allele frequency	39.3%	35.4%	34.8%	32.1%
IL-17F (rs763780)				
T/T	143	74	38	32
T/C	41	16	8	7
C/C	3	0	0	0
C allele frequency	12.7%	8.9%	9.9%	9.0%
MIF (-794 CATT)				
5/5	29	18	9	9
5/6	68	26	13	9
5/7	28	8	4	4
6/6	31	12	6	5
6/7	26	21	13	8
7/7	4	3	1	2
5-CATT frequency	41.4%	39.8%	38.0%	41.9%
6-CATT frequency	41.9%	40.3%	41.3%	36.5%
7-CATT frequency	16.7%	19.9%	20.7%	21.6%
MIF (G-173C)				
G/G	119	47	21	21
G/C	59	38	25	13
C/C	8	3	0	3
C allele frequency	20.2%	25.0%	26.1%	25.7%

FD, functional dyspepsia; EPS, epigastric pain syndrome; PDS, postprandial syndrome.

DNA was denatured at 95˚C for 3 min, followed by 35 cycles at 95˚C for 30 sec, 60 or 62˚C for 40 sec, and 72˚C for 45 sec, with a final extension at 72˚C for 5 min. Thereafter, SSCP was carried out in a similar manner as described above.

Statistical analysis. Age data were expressed as the mean ± SD. Mean ages between 2 groups were compared with the Student's t-test. Allele and genotype frequencies were calculated by direct counting. The allele counts were compared by a 2x2 table using the Chi-square test. The strength of association between allele frequencies and the disease was assessed by calculating the odds ratio (OR) and 95% confidence intervals (CI) by logistic regression analysis using genotype as a variate or the number of alleles as a co-variate. Adjusted ORs were calculated with the use of logistic regression analysis after adjustment for age, gender and *H. pylori* infection status. The interaction between *H. pylori* infection and genotype was assessed by ANOVA using *H. pylori* infection status, gender and each genotype as variates. 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 the IL-17A, -17F and MIF gene polymorphisms were clearly identified by SSCP. The characteristics of subjects and the frequencies of genotypes are shown in Table I. All genotypes were in Hardy-Weinberg equilibrium. The mean age of the non-symptom subjects was significantly lower than that of the dyspeptic subjects (Student's t-test; $p<0.0001$). The male/female ratio was significantly higher in the former than in the latter by the Chi-square test ($p=0.00035$). There were no significant differences between *H. pylori* positivity and the frequencies of all genotypes between the two groups.

Table II. Association between dyspepsia symptoms and IL-17A -17F polymorphisms.

Variables	OR (95% confidence intervals)		
	Unadjusted	Adjusted[a]	Adjusted[a]
Number of -197A alleles[b]	0.80 (0.53-1.22)	1.09 (0.66-1.79)	-
Number of 7488T alleles[b]	1.47 (0.79-2.71)	1.58 (0.76-3.29)	-
-197A carrier	0.82 (0.48-1.39)	-	1.32 (0.70-2.48)
7488T/T homozygote	1.42 (0.75-2.69)	-	1.58 (0.75-3.35)
Male gender	**0.39 (0.24-0.66)[c]**	**0.39 (0.21-0.71)[d]**	**0.37 (0.20-0.69)[e]**
H. pylori infected	0.78 (0.43-1.39)	0.83 (0.45-1.53)	0.84 (0.46-1.53)

[a]Adjustment for age, gender and *H. pylori* infection. [b]Analyses were performed using the number of each allele as a co-variate. [c]p=0.0004, [d]p=0.0021, [e]p=0.0016.

Table III. Association between dyspepsia symptoms and MIF polymorphisms.

Variables	OR (95% confidence intervals)	
	Unadjusted	Adjusted[a]
Number of 5-CATT[b]	0.94 (0.65-1.35)	1.01 (0.66-1.54)
Number of 7-CATT[b]	1.25 (0.78-2.01)	1.12 (0.65-1.95)
Number of -173C[b]	1.30 (0.84-2.03)	1.21 (0.71-2.05)
5-CATT carrier	0.71 (0.42-1.19)	0.80 (0.43-1.48)
7-CATT carrier	1.26 (0.74-2.15)	1.23 (0.65-2.31)
-173C carrier	1.55 (0.93-2.59)	1.53 (0.83-2.81)
Male gender	**0.41 (0.24-0.68)[c]**	-
H. pylori infected	0.76 (0.42-1.37)	-

[a]Adjustment for age, gender and *H. pylori* infection. [b]Analyses were performed using the number of each allele as a co-variate. [c]p=0.0007.

The association between functional dyspepsia and the IL-17A, -17F, and MIF gene polymorphisms. According to the unadjusted and adjusted analyses, female gender was significantly associated with the increased risk for FD (Tables II and III). There was no significant relationship between FD and all genotypes of the IL-17A, -17F and MIF genes.

The association between the subgroups of functional dyspepsia and the IL-17A, -17F, and MIF gene polymorphisms. Almost all of the genotypes of the IL-17A, -17F and MIF genes were not associated with the EPS and PDS subgroups (Table IV). Only the MIF -173C allele carrier had a significantly increased risk for developing EPS, a subgroup of FD, by the unadjusted and adjusted analyses (OR, 2.11; 95% CI, 1.10-4.06; p=0.025, and OR, 2.12; 95% CI, 1.00-4.49; p=0.0497, respectively).

Table IV. Association between phenotypes of functional dyspepsia and IL-17A, -17F, MIF genotypes.

Variables	OR (95% confidence intervals)			
	Epigastric pain syndrome		Postprandial syndrome	
	Unadjusted	Adjusted[a]	Unadjusted	Adjusted[a]
IL-17A, -17F				
Number of -197A[b]	0.78 (0.46-1.33)	1.13 (0.62-2.06)	0.69 (0.38-1.26)	0.92 (0.48-1.77)
Number of 7488T[b]	1.50 (0.67-3.35)	1.65 (0.65-4.21)	1.45 (0.62-3.39)	1.41 (0.54-3.73)
-197A carrier	0.71 (0.37-1.39)	1.20 (0.55-2.63)	0.78 (0.38-1.62)	1.18 (0.52-2.67)
7488T/T homozygote	1.46 (0.64-3.37)	1.64 (0.64-4.22)	1.41 (0.58-3.41)	1.45 (0.54-3.93)
MIF				
Number of 5-CATT[b]	0.87 (0.54-1.39)	0.97 (0.57-1.65)	1.02 (0.61-1.70)	1.08 (0.63-1.88)
Number of 7-CATT[b]	1.33 (0.73-2.41)	1.20 (0.61-2.34)	1.39 (0.74-2.61)	1.24 (0.63-2.45)
Number of -173C[b]	1.53 (0.88-2.66)	1.44 (0.76-2.72)	1.27 (0.71-2.29)	1.17 (0.62-2.21)
5-CATT carrier	0.63 (0.33-1.23)	0.75 (0.35-1.60)	0.72 (0.35-1.48)	0.81 (0.37-1.81)
7-CATT carrier	1.42 (0.73-2.77)	1.40 (0.65-3.05)	1.34 (0.65-2.80)	1.32 (0.58-2.98)
-173C carrier	**2.11 (1.10-4.06)[c]**	**2.12 (1.00-4.49)[d]**	1.35 (0.66-2.77)	1.35 (0.61-2.98)

[a]Adjustment for age, gender and *H. pylori* infection. [b]Analyses were performed using the number of each allele as a co-variate. [c]p=0.025, [d]p=0.0497.

Table V. Association between phenotypes of functional dyspepsia and IL-17A, -17F, and MIF genotypes in *H. pylori*-positive or -negative cases.

	OR (95% confidence intervals)			
	Epigastric pain syndrome		Postprandial syndrome	
Variables	*H. pylori* positive	*H. pylori* negative	*H. pylori* positive	*H. pylori* negative
IL-17A, -17F				
Number of -197A	1.52 (0.63-3.66)	0.77 (0.29-2.04)	1.23 (0.54-2.82)	0.56 (0.18-1.70)
Number of 7488T	**11.30 (1.23-103.2)**[a]	0.46 (0.12-1.71)	1.24 (0.38-4.07)	1.24 (0.19-7.95)
-197A carrier	1.47 (0.45-4.81)	0.84 (0.26-2.71)	2.01 (0.63-6.40)	0.65 (0.18-2.28)
7488T/T homozygote	**10.40 (1.17-92.3)**[b]	0.47 (0.13-1.77)	1.12 (0.32-3.97)	1.27 (0.19-8.32)
MIF				
Number of 5-CATT	0.77 (0.36-1.62)	1.20 (0.55-2.59)	0.93 (0.42-2.04)	1.11 (0.50-2.47)
Number of 7-CATT	1.93 (0.66-5.62)	0.83 (0.33-2.09)	1.74 (0.60-5.08)	1.03 (0.40-2.68)
Number of -173C	1.96 (0.79-4.90)	1.02 (0.40-2.61)	1.47 (0.57-3.78)	1.10 (0.43-2.82)
5-CATT carrier	0.75 (0.25-2.22)	0.74 (0.25-2.14)	0.97 (0.29-3.27)	0.58 (0.19-1.77)
7-CATT carrier	2.60 (0.82-8.25)	0.78 (0.26-2.39)	2.16 (0.61-7.64)	0.97 (0.31-3.03)
-173C carrier	**3.66 (1.19-11.3)**[c]	1.27 (0.44-3.62)	2.21 (0.64-7.66)	1.05 (0.35-3.21)

All analyses were performed after adjustment for age and gender. [a]p=0.032, [b]p=0.036, and [c]p=0.024.

Table VI. The interaction between gene polymorphisms and *H. pylori* infection to inflammatory cell infiltration.

	H. pylori positive	*H. pylori* negative	p value
Activity score			
IL-17F 7488T/T homozygote	1.27±0.75	0.17±0.43	
IL-17F 7488C carrier	0.94±1.06	0.47±0.74	**0.043**
MIF -173C carrier	1.22±0.87	0.31±0.62	
MIF-173G/G homozygote	1.20±0.96	0.17±0.45	0.660
Inflammation score			
IL-17F 7488T/T homozygote	2.02±0.55	0.56±0.65	
IL-17F 7488C carrier	1.81±0.54	0.87±0.74	**0.042**
MIF -173C carrier	2.09±0.47	0.69±0.68	
MIF-173G/G homozygote	1.91±0.59	0.58±0.69	0.730

The interaction was assessed by ANOVA using *H. pylori* infection status and genotype as variates.

The association between the subgroups of functional dyspepsia and the IL-17A, -17F, and MIF gene polymorphisms in H. pylori-infected or -uninfected subjects. When the interaction between the number of each allele and *H. pylori* infection to the development of FD was evaluated by ANOVA using *H. pylori* infection status, gender and the number of each allele as co-variates, we found that the number of IL-17F 7488T alleles was significantly associated with *H. pylori* infection in developing EPS (p=0.016). Therefore, we investigated the association between the subgroups of FD and genotypes in each *H. pylori*-positive or -negative case. As shown in Table V, the number of IL-17F 7488T alleles was positively correlated with the risk of EPS (OR, 11.3; 95% CI, 1.23-103.2; p=0.032). In addition, the IL-17F 7488 T/T homozygote and the MIF -173C allele carrier had an increased risk for the development of EPS (OR, 10.4; 95% CI, 1.17-92.3; p=0.036, and OR, 3.66; 95% CI, 1.19-11.3; p=0.024, respectively).

The interaction between H. pylori infection and genotype with inflammatory cell infiltration. The mean values of the activity score and inflammation score of IL-17F and MIF genotypes in *H. pylori*-positive or -negative cases are shown in Table VI. Analyses were performed in 142 subjects (74 and 68 from the FD and non-symptom subjects, respectively). A significant interaction was observed between *H. pylori* infection and the IL-17F genotype with an increase in both the activity and inflammation scores (p=0.043 and p=0.042, respectively), whereas not between *H. pylori* infection and MIF genotypes.

Discussion

In the present study, we investigated the association of the IL-17A, -17F and MIF gene polymorphisms with FD and its subgroups. We found that IL-17F 7488T and MIF -173C alleles were significantly associated with an increased risk for EPS, a subgroup of FD, in *H. pylori*-infected subjects.

The precise pathophysiology of FD is unknown. A heterogeneous group of pathophysiologic mechanisms has been implicated in the etiology of FD. Delayed gastric emptying (24), antral hypomotility (11), diminished gastric accommodation (25), abnormal duodenal sensitivity to acid (26), enhanced visceral sensitivity (27), and psychological factors (28) have all been identified in subgroups of patients with FD, with much overlap. However, the relationship between inflammation and clinical presentation and the treatment response is not well established in FD, although histological inflammation has been implicated in the generation of gastrointestinal pain or discomfort (29).

In the present study, we showed that MIF -173C allele carriers had a significantly increased risk for developing EPS, a subgroup of FD. In addition, the IL-17F 7488T allele, as well as the MIF -173C allele, were positively correlated with developing EPS in *H. pylori*-infected subjects. Several studies have shown that the -173C/-794 7-CATT haplotype is of importance in the susceptibility to inflammatory or immune disorders (18,19,30,31). With regard to the IL-17F polymorphism, Kawaguchi *et al* revealed that functional consequences of the H161R substitution were examined by using recombinant wild-type and mutant IL-17F proteins, and the expression and/or activity of IL-17F may be suppressed in IL-17F/7488C allele carriers (14). Thus, both MIF -173C and IL-17F 7488T alleles seem to be associated with an increased risk for inflammation. These facts suggest that polymorphisms promoting inflammation are associated with EPS of FD under the influence of *H. pylori* infection. That is, gastric mucosal inflammation may affect the pathophysiology of certain subgroups of FD. During investigation of dyspepsia, three major structural causes are readily identifiable: peptic ulcer disease (10%), gastroesophageal reflux (20%) (with or without esophagitis) and malignancy (2%) (32). This fact also suggests that one of the FD subgroup may be closely associated with gastric mucosal inflammation.

It is well known that *H. pylori* plays a major role in the pathogenesis of gastroduodenal inflammation, including gastric and duodenal ulcers. Although *H. pylori* infection has been reported to be more frequent in patients with non-ulcer dyspepsia than control populations, the role of *H. pylori* infection in functional dyspepsia is still controversial (33). Many trials evaluating the efficacy of *H. pylori* eradication treatment for FD have provided conflicting results but there is a clear indication that *H. pylori* eradication treatment is effective in at least one subset of patients with FD. A previously published meta-analysis demonstrated that *H. pylori* eradication at 12 months had a small but statistically significant benefit in the treatment of FD (34). These facts suggest that *H. pylori*-induced gastric mucosal inflammation may play an important role in the pathophysiology of FD.

It is unclear what role *H. pylori* plays in the symptom profile of the FD patient. Sarnelli *et al* reported no association between *H. pylori* infection and the overall prevalence of symptoms or gastric sensorymotor functions (35). In our study, there was no significant difference in the *H. pylori*-positive ratio among the subjects in the EPS or PDS subgroups. On the other hand, Holtmann *et al* reported that *H. pylori*-positive individuals are as likely to have symptoms of bloating and early satiety as symptoms suggestive of peptic ulcer (36). It was also reported that the abdominal pain score and indigestion score of the Gastrointestinal Symptoms Rating Scale are correlated with histological gastric mucosal atrophy and were significantly decreased after eradication therapy in *H. pylori*-infected patients with ulcer-like FD (37,38). Furthermore, Lamb *et al* demonstrated, using an experimental model, that gastric inflammation results in a hypersensitivity to chemical stimulation, including intraluminal acid, suggesting that sensitization to these stimulus modalities may contribute to the development of dyspeptic symptoms in patients with organic or functional diseases of the upper gastrointestinal tract (39). These facts indicate that *H. pylori*-induced gastric inflammation may produce the dyspeptic symptoms, including abdominal pain, via the up-regulating sensitivity to intraluminal acid. In our present study, a significant interaction was observed between the IL-17F 7488 and MIF -173 gene polymorphisms and *H. pylori* infection with the increase of neutrophil and mononuclear cell infiltration into the gastric mucosa. Therefore, we believe that the polymorphisms of the IL-17F and MIF genes affect epigastric pain in FD patients by the increased severity of *H. pylori*-induced gastric inflammation.

Recent evidence supports the relevance of a genetic milieu in FD. A case-control study by Holtmann *et al* suggested that there is a significant link between the GNß3 (C825T) CC genotype and functional dyspepsia (8). This association was independently confirmed (9). However, there are no reports suggesting the relationship between the polymorphisms of molecules associated with inflammation or immune response and FD. Depending on the population under study, between 30 and 65% of patients diagnosed with functional dyspepsia have *H. pylori*-induced gastritis (40,41). Our data also showed that the positivity of *H. pylori* infection in dyspeptic patients was 54%. Therefore, it is feasible that the polymorphisms of the genes associated with inflammation- or immune response-related molecules interact with *H. pylori*-induced gastric inflammation for the pathophysiology of one of the FD subgroup, although further studies will be necessary.

In conclusion, the IL-17F 7488T and MIF -173C alleles were significantly associated with the development of FD, particularly EPS, a subgroup of FD, in *H. pylori*-infected subjects. Our results provided the first evidence that the genetic polymorphisms of molecules associated with inflammation or immune response may be involved in the development of one of the FD subgroups via *H. pylori*-induced gastric inflammation.

Acknowledgements

This study was supported, in part, by a Grant-in-Aid for the 21st Century Center of Excellence Program of Fujita Health University from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1. Talley NJ, Stanghellini V, Heading RC, Koch KL, Malagelada JR and Tytgat GN: Functional gastro duodenal disorders. Gut 45 (suppl II): 37-42, 1999.
2. Talley NJ, Zinsmeister AR, Schleck CD and Melton LJ III: Dyspepsia and dyspepsia subgroups. Gastroenterology 102: 1259-1268, 1992.
3. Tack J, Bisschops R and Sarnelli G: Pathophysiology and treatment of fuctional dyspepsia. Gastroenterology 127: 1239-1255, 2004.
4. Tack J, Piessevaux H, Coulie B, Caenepeel P and Janssens J: Role of impaired gastric accommodation to a meal in functional dyspepsia. Gastroenterology 115: 1346-1352, 1998.
5. Lunding JA, Tefera S, Gilja OH, *et al*: Rapid initial gastric emptying and hypersensitivity to gastric filling in functional dyspepsia: effects of duodenal lipids. Scand J Gastroenterol 41: 1028-1036, 2006.
6. Chen TS, Lee YC, Chang FY, Wu HC and Lee SD: Psychosocial distress is associated with abnormal gastric myoelectrical activity in patients with functional dyspepsia. Scand J Gastroenterol 41: 791-796, 2006.
7. Locke GR III, Zinsmeister AR, Talley NJ, Fett SL and Melton LJ III: Familial association in adults with functional gastrointestinal disorders. Mayo Clin Proc 75: 907-912, 2000.
8. Holtmann G, Siffert W, Haag S, *et al*: G-protein beta 3 subunit 825 CC genotype is associated with unexplained (functional) dyspepsia. Gastroenterology 126: 971-979, 2004.
9. Camilleri CE, Carlson PJ, Camilleri M, *et al*: A study of candidate genotypes associated with dyspepsia in a U.S. community. Am J Gastroenterol 101: 581-592, 2006.
10. Tack J, Talley NJ, Camilleri M, *et al*: Functional gastroduodenal disorders. Gastroenterology 130: 1466-1479, 2006.
11. Stanghellini V, Ghidini G, Maccarini MR, Paparo GF, Corinaldesi R and Barbara L: Fasting and postprandial gastrointestinal motility in ulcer and non-ulcer dyspepsia. Gut 33: 184-190, 1992.
12. Scott AM, Kellow JE, Shuter B, *et al*: Intragastric distribution and gastric emptying of solids and liquids in functional dyspepsia. Dig Dis Sci 38: 2247-2254, 1993.
13. Jian R, Ducrot F, Ruskone A, *et al*: Symptomatic, radionuclide and therapeutic assessment of chronic idiopathic dyspepsia. A double-blind placebo controlled evaluation of cisapride. Dig Dis Sci 34: 657-664, 1989.
14. Kawaguchi M, Adachi, M, Oda, N, Kokubu, F and Huang SK: IL-17 cytokine family. J Allergy Clin Immunol 114: 1265-1274, 2004.
15. Kawaguchi M, Takahashi D, Hizawa N, *et al*: IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity. J Allergy Clin Immunol 117: 795-801, 2006.
16. Bloom BR and Bennett B: Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. Science 153: 80-82, 1966.
17. Calandra T, Echtenacher B, Roy DL, *et al*: Protection from septic shock by neutralization of macrophage migration inhibitory factor. Nat Med 6: 164-170, 2000.
18. Donn R, Alourfi Z, De Benedetti F, *et al*: Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. Arthritis Rheum 46: 2402-2409, 2002.
19. Amoli MM, Donn RP, Thomson W, *et al*: Macrophage migration inhibitory factor gene polymorphism is associated with sarcoidosis in biopsy proven erythema nodosum. J Rheumatol 29: 1671-1673, 2002.
20. Luzza F, Parrello T, Monteleone G, *et al*: Up-regulation of IL-17 is associated with bioactive IL-8 expression in *Helicobacter pylori*-infected human gastric mucosa. J Immunol 165: 5332-5337, 2000.
21. Xia HHX, Lam SK, Huang XR, *et al*: *Helicobacter pylori* infection is associated with increased expression of macrophage migration inhibitory factor by epithelial cells, T cells and macrophages in gastric mucosa. J Infect Dis 190: 293-302, 2004.
22. Arisawa T, Tahara T, Shibata T, *et al*: The relationship between *Helicobacter pylori* infection and promoter polymorphism of the Nrf2 gene in chronic gastritis. Int J Mol Med 19: 143-148, 2007.
23. Arisawa T, Tahara T, Shibata T, *et al*: Association between genetic polymorphisms in cyclooxygenase-1 gene promoter and peptic ulcer in Japan. Int J Mol Med 20: 373-378, 2007.
24. Greydanus MP, Vassallo M, Camilleri M, Nelson DK, Hanson RB and Thomforde GM: Neurohormonal factors in functional dyspepsia: insights on pathophysiological mechanisms. Gastroenterology 100: 1311-1318, 1991.
25. Gilja OH, Hausken T, Wilhelmsen I and Berstad A: Impaired accommodation of proximal stomach to a meal in functional dyspepsia. Dig Dis Sci 41: 689-696, 1996.
26. Samsom M, Verhagen MA, vanBerge Henegouwen GP and Smout AJ: Abnormal clearance of exogenous acid and increased acid sensitivity of the proximal duodenum in dyspeptic patients. Gastroenterology 116: 515-520, 1999.
27. Lemann M, Dederding JP, Flourie B, Franchisseur C, Rambaud JC and Jian R: Abnormal perception of visceral pain in response to gastric distension in chronic idiopathic dyspepsia. The irritable stomach syndrome. Dig Dis Sci 36: 1249-1254, 1991.
28. Soo S, Forman D, Delaney BC and Moayyedi P: A systematic review of psychological therapies for nonulcer dyspepsia. Am J Gastroenterol 99: 1817-1822, 2004.
29. Collins SM: The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. Gastroenterology 111: 1683-1699, 1996.
30. Baugh JA, Chitnis S, Donnelly SC, *et al*: A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. Genes Immun 3: 170-176, 2002.
31. Hizawa N, Yamaguchi E, Takahashi D, Nishihara J and Nishimura M: Functional polymorphisms in the promoter region of macrophage migration inhibitory factor and atopy. Am J Respir Crit Care Med 169: 1014-1018, 2004.
32. Talley NJ, Vakil NB and Moayyedi P: American Gastroenterological Association Technical Review on the Evaluation of Dyspepsia. Gastroenterology 129: 1756-1780, 2005.
33. McColl K, Murray L, El-Omar E, *et al*: Symptomatic benefit from eradicating *Helicobacter pylori* infection in patients with nonulcer dyspepsia. N Engl J Med 339: 1869-1874, 1998.
34. Moayyedi P, Deeks J, Talley NJ, Delaney B and Forman D: An update of the Cochrane systematic review of *Helicobacter pylori* eradication therapy in nonulcer dyspepsia: resolving the discrepancy between systematic reviews. Am J Gastroenterol 98: 2621-2626, 2003.
35. Sarnelli G, Cuomo R, Janssens J and Tack J: Symptom patterns and pathophysiological mechanisms in dyspeptic patients with and without *Helicobacter pylori*. Dig Dis Sci 48: 2229-2236, 2003.
36. Holtmann G, Stanghellini V and Talley NJ: Nomenclature of dyspepsia, dyspepsia subgroups and functional dyspepsia: clarifying the concepts. Baillieres Clin Gastroenterol 12: 417-433, 1998.
37. Kadouchi K, Tominaga K, Ochi M, *et al*: Interactions between the grading of gastric atrophy associated with *Helicobacter pylori* infection and the severity of clinical symptoms and delay in gastric emptying in patients with functional dyspepsia. Aliment Pharmacol Ther 24 (suppl 4): 49-57, 2006.
38. Suzuki H, Masaoka T, Sakai G, Ishii H and Hibi T: Improvement of gastrointestinal quality of life scores in cases of *Helicobacter pylori*-positive functional dyspepsia after successful eradication therapy. J Gastroenterol Hepatol 20: 1652-1660, 2005.
39. Lamb K, Kang YM, Gerald GF and Bielefeldt K: Gastric inflammation triggers hypersensitivity to acid in awake rats. Gastroenterology 125: 1410-1418, 2003.
40. Talley NJ: *Helicobacter pylori* and non-ulcer dyspepsia. Scand J Gastroenterol (Suppl) 220: 19-22, 1996.
41. Armstrong D: *Helicobacter pylori* infection and dyspepsia. Scand J Gastroenterol (Suppl) 215: 38-47, 1996.