A Genetic Variant of the CD14 C-159T in Patients with Functional Dyspepsia (FD) in Japanese Subjects

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Summary 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. CD14 is an important mediator of the inflammatory response in the first line of host defense by recognition of Lipopolysaccharide (LPS). We aimed to investigate the association between CD14 promoter C-159T polymorphism and FD in a Japanese population. 108 patients with FD and 99 non-dyspeptic subjects enrolled in this study. Dyspeptic symptoms were divided according to Rome III criteria. CD14 gene C-159T polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism. In the non-dyspeptics, the CD14 genotype distribution was 28CC (28.3%), 51CT (51.5%), 21TT (21.2%). Meanwhile, the CD14 genotype distribution in FD was 31CC (28.4%), 56CT (51.4%), 22TT (20.2%). The genotype distribution was not significantly different. There was no significant difference between two groups in the genotype distribution. We did not find any association between CD14 genotypes and dyspeptic patients in different gender and *Helicobacter pylori* infection status. No significant association was also found between CD14 polymorphism and any of different subtypes of FD according to Rome III while there was a weak correlation between TT genotype and PDS in male subjects (TT vs others, OR = 3.18, 95% CI = 0.98–10.26, $p = 0.06$). In conclusion, our results suggest that CD14 polymorphism is unlikely to associate with susceptibility of dyspeptic symptoms. The role of inflammation related-gene polymorphisms to the development of dyspepsia needs to further evaluation.

Key Words: functional dyspepsia, CD14, polymorphism

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 abnormality identifiable by conventional diagnostic tests, including upper gastrointestinal endoscopy [1]. Talley *et al.* have shown that up to 25% of the population experienced these symptoms [2]. FD is a heterogeneous condition indicated by the variety of different pathophysiologic mechanisms that have been demonstrated in this disorder [3], so FD does not have a well pathophysiology. Gastrointestinal motor

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abnormalities [4], altered visceral sensation [5] and psychosocial factors [6] have thought to be essential in the pathophysiology of FD. Recently, Locke *et al.* reported familial clustering of FD [7]. In addition, it has been reported that G-protein β_3 subunit gene polymorphism was associated with FD [8, 9]. These facts suggest that the genetic factor 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 this organism infection 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 gastroduodenal mucosa by gastrointestinal endoscopy were diagnosed as FD. That is, there is a possibility that one of the FD subgroups may relate to the gastric mucosal inflammation, although adult FD patients frequently have motility abnormalities of the stomach and upper small bowel including antral hypomotility and delayed gastric emptying [11–13].

CD14 is a important mediator of the inflammatory response in the first line of host defense by recognition of Lipopolysaccharide (LPS). LPS, a major component of the outer cell wall of gram-negative bacteria, is a potent and well-characterized inducer of inflammation [14]. After forming a complex with the LPS-binding protein, LPS interacts with a membrane CD14 [15, 16]. This complex induces second-messenger and signal transduction pathways [17]. These signals in turn activate transcription factors, mainly nuclear factor- κ B and cytokines [15, 18].

Recently it was reported that soluble CD14 levels tended to be higher in *H. pylori* positives than in *H. pylori* negatives [19]. Furthermore, CD14 showed a trend to be increased in FD patients with extensive gastric inflammation and high density of *H. pylori* [20]. These findings support a sentinel role for CD14 in the mucosal inflammation response to *H. pylori*.

A polymorphism in the promoter region of the CD14 gene has been described. This polymorphism involves a C-T substitution at base-pair -159 of the 5' flanking region of the CD14 gene [21]. Genotypes include the CC homozygote, the CT heterozygote, and the TT homozygote. The TT homozygote has been associated with increased circulating soluble CD14 levels and a higher density of the monocyte CD14 receptor [22].

Because of the important roles that CD14 play with respect to LPS binding and signaling, we hypothesized that polymorphisms in the CD14 gene promoter may affect the severity of gastric mucosal inflammation and modify the risk of FD. In the present study, we investigated the prevalence of this polymorphism in patients with FD diag-

nosed according to the Roma III criteria in a Japanese population. We also wished to assess its effect on the different subtypes of FD.

Materials and Methods

Study populations

We studied 207 subjects attending the Endoscopy Center of Fujita Health University Hospital from January 2005 to October 2006. The subjects underwent upper gastroscopy for their health check, secondary complete check up of stomach cancer following to barium X ray examination, or for the complaint of abdominal discomfort. Subjects who have significant upper gastrointestinal findings such as peptic ulcer disease, reflux esophagitis and malignancies were excluded from this study. Patients with malignancies in other organs, and had received non-steroidal anti-inflammatory drugs, antibiotics, and *H. pylori* eradication treatment were also excluded. Other diseases were also excluded by face-to-face history and physical examination including blood test, abdominal US and ECG. According to the Roma III criteria, 108 FD 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 upper abdomen. In 108 dyspeptic patients, 44 and 30 patients were diagnosed as epigastric pain syndrome (EPS) and postprandial syndrome (PDS) respectively. Subjects who had no dyspeptic symptoms within the last 12 months were considered as non-dyspepsia subjects. Subjects who were negative by upper gastroscopy and negative for dyspeptic symptom with in last 12 months were considered as healthy controls. Those who had received or proton-pump inhibitory drugs or H2RAS during the 4 week were excluded from healthy controls. The Ethics Committee of Fujita Health University School of Medicine approved the protocol and written informed consent was obtained from all of the subjects.

Detection of *H. pylori* infection

The *H. pylori* infection status was determined on the basis of histology, culture, the rapid urease test (RUT), and antibodies to *H. pylori*. Infection was diagnosed when at least one of these 4 tests was positive.

Genotyping for CD14 gene

Genomic DNA was extracted from non-pathological gastric biopsies or peripheral blood using the standard phenol/chloroform method. Then the genotyping for C-159T of CD14 was determined using the methods described by Hubacek *et al.* [22]. The promoter of the CD14 was amplified by the primers 5'-ttggtgccacagatgaggttcac-3' and 5'-ttcttctctacacagtgggaccc-3' under the following conditions: an initial denaturation at 95°C for 5 min, followed by 38

Table 1. Characteristics of subjects

	Dyspeptics	Non-dyspeptics	<i>p</i>
Subjects (n)	108	99	
Sex [male/female (%/%)]	45/64 (42/58)	61/38 (62/38)	0.004\$
Mean age $\pm$ SD (y)	59.59 $\pm$ 13.15	60.39 $\pm$ 13.45	0.49*
<i>H. pylori</i> infection positive ratio (%)	53.7	61.6	0.26\$

\$; χ^2 test *; Mann-Whitney *U* test

Table 2. CD14 C-159T polymorphism and risk of FD

Variables n (%)	genotype/n (%)			OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
	CC	CT	TT								
Over all controls (99)	28 (28.3)	51 (51.5)	21 (21.2)	Reference		Reference		Reference		Reference	
Over all FD (109)	31 (28.4)	56 (51.4)	22 (20.2)	0.95 (0.43–2.08)	1	0.99 (0.52–1.87)	1	0.98 (0.54–1.79)	1	0.94 (0.48–1.84)	1
EPS (44)	15	30	10	0.89 (0.33–2.37)	1	1.10 (0.51–2.38)	0.85	1.04 (0.19–1.59)	1	0.84 (0.36–1.93)	0.83
PDS (30)	11	14	12	1.45 (0.54–3.93)	0.61	0.60 (0.23–1.53)	0.48	1.07 (0.50–2.17)	0.83	1.81 (0.78–4.18)	0.18
Others (17)	7	16	3	0.57 (0.13–2.48)	0.51	0.24 (0.05–0.98)	0.8	1.06 (0.40–2.79)	1	0.49 (0.13–1.79)	0.4

Statistical analysis was performed by two-sided Fisher's exact test.

cycles of 95°C for 40 s, 60°C for 30 s, and 72°C for 40 s. The final extension step was prolonged to 7 min. The 561 bp PCR product (10 μ l) was cleaved by *Ava*II (New England Biolabs. Inc, Beverly, MA) in an appropriate buffer at 37°C overnight. The product was digested into the fragments of 204 bp, 201 bp and 156 bp length in the presence of wild type allele. The variant allele showed a loss of one cleavage site resulting in the presence of fragments 360 and 201 bp in length.

Statistical analysis

Hardy-Weinberg equilibrium of the CD14 gene allele in the healthy controls and dyspeptic patients were assessed by χ^2 statistics. Differences of CD14 genotype frequencies among two groups were determined by the two-sided Fisher's exact test. The odds ratio (OR) and 95% confidence interval (CI) were also calculated. A probability value of less than 0.05 was considered statistically significant in all analyses.

Results

Study population

A total of 108 dyspeptic patients, 99 non-symptomatic healthy controls participated in this study. The characteristics of the subjects are summarized in Table 1. Age and *H. pylori* infection positive ratios were not significantly different among those two groups but female sex ratio was significantly higher in the dyspeptic patients than those of non-symptomatic healthy controls.

CD14 genotypes

C-159T polymorphism of CD14 was investigated in all 207 subjects. The frequency of CD14 polymorphism in the non-symptomatic healthy controls and FD did not deviate significantly from those expected under the Hardy-Weinberg equilibrium ($p = 0.80, 0.72$ respectively). In the non-symptomatic healthy controls, the CD14 genotype distribution was 28CC (28.3%), 51CT (51.5%), 21TT (21.2%). Meanwhile, the CD14 genotype distribution in FD was 31CC (28.4%), 56CT (51.4%), 22TT (20.2%). The genotype distribution was not significantly different (Table 2).

To further evaluate the effect of CD14 gene polymorphism on dyspeptic symptom, we also investigated the prevalence of CD14 gene polymorphism in different gender and *H. pylori* infection status. However, we did not find any association between CD14 genotype and FD in different gender and *H. pylori* infection status (Table 3). We also investigated the prevalence of CD14 polymorphism in different subtypes of FD according to Rome III. Although there was a weak correlation between TT genotype and PDS in male subjects (TT vs others, OR = 3.18, 95%CI = 0.98–10.26, $p = 0.06$), no significant association was also found probably due to the small number of each subtypes (Tables 2 and 3).

Discussion

The precise pathophysiology of FD is unknown. A heterogeneous group of pathophysiologic mechanisms have been implicated in the etiology of FD. Delayed gastric emptying [23], antral hypomotility [24], diminished gastric accommo-

Table 3. Association between CD14 C-159T polymorphism and clinicopathological feature of FD

Variables n (%)	genotype/n			OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>	OR (95% CI)	<i>p</i>
	CC	CT	TT	CC vs TT		CC vs CT		CC vs T carriers		TT vs others	
<i>Hp (–)</i>											
Controls (37)	9	19	10	Reference		Reference		Reference		Reference	
FD (50)	17	24	9	0.48 (0.14–1.60)	0.36	0.67 (0.24–1.83)	0.46	0.60 (0.23–1.56)	0.35	0.61 (0.20–1.71)	0.44
EPS (29)	10	15	4	0.36 (0.08–1.56)	0.29	0.71 (0.23–2.19)	0.58	0.59 (0.20–1.72)	0.42	0.45 (0.12–1.61)	0.24
PDS (19)	8	6	5	0.56 (0.13–2.36)	0.49	0.36 (0.09–1.33)	0.18	0.43 (0.13–1.39)	0.22	1.00 (0.29–3.49)	1
Others (8)	1	6	1	0.90 (0.05–16.59)	1	2.84 (0.29–27.25)	0.64	2.17 (0.23–20.10)	0.66	0.40 (0.04–3.67)	0.66
<i>HP (+)</i>											
Controls (61)	19	31	11	Reference		Reference		Reference		Reference	
FD (59)	14	32	13	1.60 (0.55–4.62)	0.43	1.40 (0.60–3.27)	0.52	1.45 (0.65–3.26)	0.42	1.28 (0.52–3.15)	0.65
EPS (21)	5	15	5	1.73 (0.41–7.33)	0.48	1.84 (0.58–5.88)	0.41	1.81 (0.59–5.54)	0.27	1.14 (0.35–3.69)	1
PDS (14)	3	8	6	3.45 (0.72–16.64)	0.14	1.63 (0.39–6.93)	0.73	2.11 (0.54–8.22)	0.37	2.48 (0.75–8.15)	0.18
Others (13)	6	10	2	0.58 (0.10–3.36)	0.69	1.02 (0.32–3.27)	1	0.90 (0.30–2.77)	1	0.57 (0.11–2.84)	0.72
<i>Male</i>											
Controls (61)	18	31	12	Reference		Reference		Reference		Reference	
FD (45)	11	24	11	1.50 (0.49–4.55)	0.58	1.27 (0.50–3.18)	0.65	1.33 (0.56–3.19)	0.66	1.28 (0.51–3.24)	0.64
EPS (21)	7	11	3	0.64 (0.14–2.99)	0.71	0.91 (0.30–2.77)	1	0.84 (0.29–2.42)	0.79	0.68 (0.17–2.69)	0.75
PDS (16)	3	6	7	3.50 (0.75–16.28)	0.15	1.16 (0.26–5.23)	1	1.81 (0.46–7.14)	0.53	3.18 (0.98–10.26)	0.06
Others (9)	1	7	1	1.50 (0.09–26.36)	1	4.06 (0.46–35.75)	0.25	3.35 (0.39–28.76)	0.43	0.51 (0.06–4.48)	1
<i>Female</i>											
Controls (38)	10	19	9	Reference		Reference		Reference		Reference	
FD (64)	20	33	11	0.61 (0.19–1.96)	0.55	0.87 (0.34–2.24)	0.81	0.79 (0.32–1.92)	0.66	0.67 (0.25–1.80)	0.45
EPS (32)	8	18	6	0.83 (0.21–3.34)	1	1.18 (0.38–3.67)	1	1.07 (0.36–3.15)	1	0.74 (0.23–2.37)	0.77
PDS (20)	8	8	4	0.56 (0.12–2.49)	0.48	0.53 (0.15–1.83)	0.35	0.54 (0.17–1.69)	0.37	0.81 (0.21–3.04)	1
Others (18)	6	10	2	0.37 (0.06–2.32)	0.4	0.88 (0.25–3.12)	1	0.71 (0.21–2.41)	0.79	0.40 (0.08–2.10)	0.47
<i>Different generation</i>											
<i><60</i>											
Controls (37)	7	19	11	Reference		Reference		Reference		Reference	
FD (48)	14	25	9	0.41 (0.12–1.45)	0.21	0.66 (0.26–1.64)	0.59	0.59 (0.20–1.59)	0.32	0.55 (0.20–1.50)	0.3
EPS (25)	8	14	3	0.24 (0.05–1.22)	0.13	0.64 (0.19–2.20)	0.54	0.5 (0.15–1.61)	0.36	0.32 (0.08–1.30)	0.13
PDS (15)	5	5	5	0.64 (0.13–3.03)	0.7	0.37 (0.08–1.67)	0.25	0.47 (0.12–1.80)	0.29	1.18 (0.33–4.27)	0.77
Others (11)	2	8	1	0.32 (0.02–4.20)	0.55	1.47 (0.25–8.70)	1	1.05 (0.18–5.38)	1	0.24 (0.03–2.08)	0.25
<i>60>=</i>											
Controls (62)	21	31	10	Reference		Reference		Reference		Reference	
FD (62)	18	31	13	1.52 (0.54–4.28)	0.6	1.17 (0.52–2.60)	0.84	1.25 (0.59–2.68)	0.7	1.38 (0.55–3.43)	0.64
EPS (29)	7	16	6	1.80 (0.48–6.77)	0.5	1.55 (0.54–4.41)	0.45	1.61 (0.59–4.38)	0.79	1.36 (0.44–4.18)	0.57
PDS (21)	6	9	6	2.10 (0.54–8.18)	0.31	1.02 (0.31–3.28)	1	1.28 (0.43–3.78)	0.79	2.08 (0.65–6.66)	0.22
Others (16)	6	8	2	0.70 (0.12–4.10)	1	0.90 (0.27–2.98)	1	0.85 (0.27–2.67)	0.78	0.74 (0.15–3.79)	1

Statistical analysis was performed by two-sided Fisher's exact test.

dation [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 treatment response is not well established in FD, although histological inflammation has been implicated in the generation of gastrointestinal pain or discomfort [29].

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%) [30]. It is well known that *H. pylori* plays a major role in the pathogenesis of gastro-duodenal

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 population, the role of *H. pylori* infection in functional dyspepsia is still controversial [31]. Many trials evaluating the efficacy of *H. pylori* eradication treatment for FD have given conflicting results but there is a clear indication that *H. pylori* eradication treatment is effective in at least a subset of patients with FD. The recently published meta-analysis suggests that *H. pylori* eradication at 12 months has a small but statistically significant benefit in the treatment of FD [32]. These facts suggest that *H. pylori*-induced gastric mucosal inflammation may play an important role on the

pathophysiology of FD.

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 GN β 3 (C825T) CC genotype and functional dyspepsia [8]. The association has been independently confirmed [9]. However, there are no reports demonstrated the relationship between the polymorphisms of molecules associated with inflammation and FD. Depending on the population under study, between 30%–65% of patients diagnosed with functional dyspepsia has *H. pylori*-induced gastritis [33, 34]. Our data in the present study also showed that the positivity of *H. pylori* infection in dyspeptic patients was 53.7%. Therefore, it is feasible that polymorphisms of the inflammation-related molecule genes may interact with *H. pylori*-induced gastric inflammation to the pathophysiology of one of the FD subgroup.

In the present study, we investigated the association of the C-159T of CD14 gene polymorphisms with FD and its subgroups because CD14 have a sentinel role for mucosal inflammation response to *H. pylori*. It has been reported that the transcriptional activity of the CD14-159T allele was found, by the luciferase reporter assay, to be increased in monocytic Mono Mac 6 cells [35]. Homozygous carriers of the T allele also have a significant increase in serum level of soluble CD14 and correlation with high IFN γ , which is produced by T helper 1 (Th1) like cell [21].

It is well known that a polarized Th1 immune response occurs in *H. pylori* infection [36] and Th1 dominant immune response has been reported to cause gastric atrophy and metaplasia. It is also shown that long standing gastric mucosal inflammation by *H. pylori* infection often leads to low acid secretion in Japanese [37]. Recently, it has been reported that intraduodenal administered acid affects gastroduodenal motility as well as sensation and the role of gastric acid in functional dyspepsia has been suggested [38, 39]. Therefore, it is reasonable to speculate that, not only by gastric inflammation, but also by altering gastric acid secretion, the CD14 polymorphism may affect the susceptibility of FD.

There was, however, no association of CD14 C-159T and development of FD. The frequencies of CD14 genotypes were not significantly different between FD and controls in 207 subjects. We further investigated the prevalence of CD14 gene polymorphism in different gender and *H. pylori* infection status, no significant association of CD14 C-159T polymorphism and FD were also found in these subgroups excluding the weak correlation between TT genotype and PDS in male subjects.

Recently, other polymorphism have been described in the CD14 gene that remained to be studied in FD [40]. In addition, the frequency of the C-159T allele may show variations in different ethnic groups. Furthermore, it has

been also reported that the GN β 3-C825T polymorphism which is associated with FD [8, 9] is not associated significantly with lower FD [41], suggesting that the relationship between FD and gene polymorphism has not been completely cleared yet.

Further genetic studies including other polymorphisms in CD14 gene will be needed in a larger and ethnically diverse population to resolve the impact of the CD14 polymorphisms in the susceptibility of FD.

In conclusion, we have shown that C-159T polymorphisms of CD14 is not associated with FD in Japanese. The present study is the first investigating a potential relationship between the C-159T polymorphism of CD14 gene and FD. Despite several potential limitations, we believe that our study provides a salient finding even if it was a negative one.

References

- [1] Talley, N.J., Stanghellini, V., Heading, R.C., Koch, K.L., Malagelada, J.R., and Tytgat, G.N.: Functional gastro duodenal disorders. *Gut*, **45** Suppl II, II37–II42, 1999.
- [2] Talley, N.J., Zinsmeister, A.R., Schleck, C.D., and Melton, L.J. 3rd.: Dyspepsia and dyspepsia subgroups. *Gastroenterology*, **102**, 1259–1268, 1992.
- [3] Tack, J., Bisschops, R., and Sarnelli, G.: Pathophysiology and treatment of functional 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, J.A., Tefera, S., Gilja, O.H., Hausken, T., Bayati, A., Rydholm, H., Mattsson, H., and Berstad, A.: 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, T.S., Lee, Y.C., Chang, F.Y., Wu, H.C., and Lee, S.D.: Psychosocial distress is associated with abnormal gastric myoelectrical activity in patients with functional dyspepsia. *Scand. J. Gastroenterol.*, **41**, 791–796, 2006.
- [7] Locke, G.R. 3rd., Zinsmeister, A.R., Talley, N.J., Fett, S.L., and Melton, L.J. 3rd.: Familial association in adults with functional gastrointestinal disorders. *Mayo Clin. Proc.*, **75**, 907–912, 2000.
- [8] Holtmann, G., Sifert, W., Haag, S., Mueller, N., Langkafel, M., Senf, W., Zott, R., and Talley, N.J.: G-protein β 3 subunit 825 CC genotype is associated with unexplained (functional) dyspepsia. *Gastroenterology*, **126**, 971–979, 2004.
- [9] Camilleri, C.E., Carlson, P.J., Camilleri, M., Castillo, E.J., Locke, G.R., Geno, D.M., Stephens, D.A., Zinsmeister, A.R., and Urrutia, R.: A study of candidate genotypes associated with dyspepsia in a U.S. community. *Am. J. Gastroenterol.*, **101**, 581–592, 2006.

- [10] Tack, J., Talley, N.J., Camilleri, M., Holtmann, G., Hu, P., Malagelada, J.R., and Stanghellini, V.: Functional gastroduodenal disorders. *Gastroenterology*, **130**, 1466–1479, 2006.
- [11] Stanghellini, V., Ghidini, G., Maccarini, M.R., Paparo, G.F., Corinaldesi, R., and Barbara, L.: Fasting and postprandial gastrointestinal motility in ulcer and non-ulcer dyspepsia. *Gut*, **33**, 184–190, 1992.
- [12] Scott, A.M., Kellow, J.E., Shuter, B., Cowan, H., Corbett, A.M., Riley, J.W., Lunzer, M.R., Eckstein, R.P., Höschl, R., and Lam, S.K.: Intra-gastric distribution and gastric emptying of solids and liquids in functional dyspepsia. *Dig. Dis. Sci.*, **38**, 2247–2254, 1993.
- [13] Jian, R., Ducrot, F., and Ruskone, A.: 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] Rietschel, E.T., Kirikae, T., Schade, F.U., Mamat, U., Schmidt, G., Loppnow, H., Ulmer, A.J., Zahring, U., Seydel, U., and Di Padova, F.: Bacterial endotoxin: molecular relationships of structure to activity and function. *FASEB J.*, **8**, 217–225, 1994.
- [15] Ulevitch, R.J. and Tobias, P.S.: Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu. Rev. Immunol.*, **13**, 437–457, 1995.
- [16] Wright, S.D., Ramos, R.A., Tobias, P.S., Ulevitch, R.J., and Mathison, J.C.: CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science*, **249**, 1431–1433, 1990.
- [17] Janeway, C.A. Jr and Medzhitov, R.: Introduction: the role of innate immunity in the adaptive immune response. *Semin. Immunol.*, **5**, 349–350, 1998.
- [18] Sweet, M.J. and Hume, D.A.: Endotoxin signal transduction in macrophages. *J. Leukoc. Biol.*, **60**, 8–26, 1996.
- [19] Karhukorpi, J., Yan, Y., Niemela, S., Valtonen, J., Koistinen, P., Joensuu, T., Saikku, P., and Karttunen, R.: Effect of CD14 promoter polymorphism and *H. pylori* infection and its clinical outcomes on circulating CD14. *Clin. Exp. Immunol.*, **128**, 326–332, 2002.
- [20] Andersen, L.P., Holck, S., Janulaityte-Günther, D., Kupcinkas, L., Kiudelis, G., Jonaitis, L., Janciauskas, D., Holck, P., Bennedsen, M., Permin, H., Norn, S., and Wadström, T.: Gastric inflammatory markers and interleukins in patients with functional dyspepsia, with and without *Helicobacter pylori* infection. *FEMS Immunol. Med. Microbiol.*, **44**, 233–238, 2005.
- [21] Baldini, M., Lohman, I.C., Halonen, M., Erickson, R.P., Holt, P.G., and Martinez, F.D.: A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am. J. Respir. Cell Mol. Biol.*, **20**, 976–983, 1999.
- [22] Hubacek, J.A., Rothe, G., Pit'ha, J., Skodova, Z., Stanek, V., Poledne, R., and Schmitz, G.: C(-260)→T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. *Circulation*, **99**, 3218–3220, 1999.
- [23] Greydanus, M.P., Vassallo, M., Camilleri, M., Nelson, D.K., Hanson, R.B., and Thomforde, G.M.: Neurohormonal factors in functional dyspepsia: insights on pathophysiological mechanisms. *Gastroenterology*, **100**, 1311–1318, 1991.
- [24] Stanghellini, V., Ghidini, C., Maccarini, M.R., Paparo, G.F., Corinaldesi, R., and Barbara, L.: Fasting and postprandial gastrointestinal motility in ulcer and non-ulcer dyspepsia. *Gut*, **33**, 184–190, 1992.
- [25] Gilja, O.H., 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, M.A., vanBerge, Henegouwen, G.P., and Smout, A.J.: 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, J.P., Flourie, B., Franchisseur, C., Rambaud, J.C., 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, B.C., and Moayyedi, P.: A systematic review of psychological therapies for nonulcer dyspepsia. *Am. J. Gastroenterol.*, **99**, 1817–1822, 2004.
- [29] Collins, S.M.: The immunomodulation of enteric neuromuscular function: implications for motility and inflammatory disorders. *Gastroenterology*, **111**, 1683–1699, 1996.
- [30] Talley, N.J., Vakil, N.B., and Moayyedi, P.: American gastroenterological association technical review on the evaluation of dyspepsia. *Gastroenterology*, **129**, 1756–1780, 2005.
- [31] McColl, K., Murray, L., El-Omar, E., Dickson, A., El-Nujumi, A., Wirz, A., Kelman, A., Penny, C., Knill-Jones, R., and Hilditch, T.: Symptomatic benefit from eradicating *Helicobacter pylori* infection in patients with nonulcer dyspepsia. *N. Engl. J. Med.*, **339**, 1869–1874, 1998.
- [32] Moayyedi, P., Deeks, J., Talley, N.J., 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.
- [33] Talley, N.J.: *Helicobacter pylori* and non-ulcer dyspepsia. *Scand. J. Gastroenterol.*, Suppl **220**, 19–22, 1996.
- [34] Armstrong, D.: *Helicobacter pylori* infection and dyspepsia. *Scand. J. Gastroenterol.*, Suppl **215**, 38–47, 1996.
- [35] LeVan, T.D., Bloom, J.W., Bailey, T.J., Karp, C.L., Halonen, M., Martinez, F.D., and Vercelli, D.: A common single nucleotide polymorphism in the CD14 promoter decreases the affinity of Sp protein binding and enhances transcriptional activity. *J. Immunol.*, **167**, 5838–5844, 2001.
- [36] Bamford, K.B., Fan, X., Crowe, S.E., Leary, J.F., Gourley, W.K., Luthra, G.K., Brooks, E.G., Graham, D.Y., Reyes, V.E., and Ernst, P.B.: Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. *Gastroenterology*, **114**, 482–492, 1998.
- [37] Kapadia, C.R.: Gastric atrophy, metaplasia, and dysplasia: a clinical perspective. *J. Clin. Gastroenterol.*, Suppl **36**, S29–36, 2003.
- [38] Lee, K.J., Vos, R., Janssens, J., and Tack, J.: Influence of duodenal acidification on the sensorimotor function of the

- proximal stomach in humans. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **286**, G278–G284, 2004.
- [39] Schwartz, M.P., Samsom, M., and Smout, A.J.: Chemo-specific alterations in duodenal perception and motor response in functional dyspepsia. *Am. J. Gastroenterol.*, **96**, 2596–26023, 2001.
- [40] Zhao, D., Sun, T., Zhang, X., Guo, Y., Yu, D., Yang, M., Tan, W., Wang, G., and Lin, D.: Role of CD14 promoter polymorphisms in *Helicobacter pylori* infection—related gastric carcinoma. *Clin. Cancer Res.*, **13**, 2362–2368, 2007.
- [41] Andresen, V., Camilleri, M., Kim, H.J., Stephens, D.A., Carlson, P.J., Talley, N.J., Saito, Y.A., Urrutia, R., and Zinsmeister, A.R.: Is there an association between GNBeta3-C825T genotype and lower functional gastrointestinal disorders? *Gastroenterology*, **130**, 1985–1994, 2006.