Association between interleukin-1β and tumor necrosis factor-α polymorphisms and symptoms of dyspepsia

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Abstract. Interleukin (IL)-1β, and tumor necrosis factor (TNF)-α have significant roles in the mediation of inflammatory immune responses and are also potent inhibitors of gastric acid secretion in the stomach. The present study aimed to investigate the associations between polymorphisms at position -31 (T>C) of the IL-1β gene and -857 (C>T) of the TNF-α gene with dyspeptic symptoms. Polymorphisms at position -31 (T>C) of the IL-1β gene and -857 (C>T) of the TNF-α gene were genotyped in 261 subjects, including 126 subjects without symptoms and 135 subjects exhibiting symptoms of dyspepsia. The IL-1β -31 CC genotype was inversely associated with dyspeptic symptoms in all subjects, as determined by the Fisher's exact test [odds ratio (OR), 0.57; 95% confidence interval (CI), 0.34-0.96; P=0.046]; however, this association was not detected following logistic regression analysis. Within the subgroups of symptoms, the CC genotype was also inversely associated with upper abdominal pain (OR, 0.28; 95% CI, 0.12-0.67; P=0.003) and epigastric pain syndrome (EPS)-like symptoms (OR, 0.14; 95% CI, 0.07-0.28; P=0.003), according to the Rome III classifications. These associations were also found following logistic regression analysis (upper abdominal pain: OR, 0.34; 95% CI, 0.14-0.80; P=0.014; and EPS-like symptoms: OR, 0.41; 95% CI, 0.20-0.84; P=0.015). No significant associations were identified between the TNF-α -857 polymorphism and dyspeptic symptoms, including amongst the various subtypes analyzed. In conclusion, the IL-1β -31 CC genotype was inversely associated with susceptibility to dyspeptic symptoms, in particular, upper abdominal pain and EPS-like symptoms.

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Abbreviations: FD, functional dyspepsia; *H. pylori*, *Helicobacter pylori*; EPS, epigastric pain syndrome; PDS, postprandial distress syndrome; Il-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; PCR, polymerase chain reaction; RFLP, restricted fragment length polymorphism

Key words: interleukin-1β, tumor necrosis factor-α, polymorphism, dyspepsia, functional dyspepsia

Introduction

The most common cause underlying the development of symptoms of dyspepsia in the West, and increasingly in other parts of the world, is functional dyspepsia (FD) (1), which affects ~25% of the population worldwide (2). Symptoms of dyspepsia are defined by the presence of 'chronic or recurrent symptoms centered in the upper abdomen in the absence of any organic, systemic or metabolic disease that is likely to explain the symptoms' (3).

FD is a heterogeneous disorder, which lacks a well-defined pathophysiology. Gastrointestinal motor abnormalities (4,5), altered visceral sensation (6,7) and psychosocial factors (8,9) have been investigated as potential pathophysiological mechanisms underlying the development of FD, and their altered functions may be partially explained by genetic abnormalities (10-12). A study reporting familial clustering of FD suggested that genetic factors may exhibit a significant role in mediating the development of FD (13).

Infection with *Helicobacter pylori (H. pylori)* represents a significant pathogenic factor underlying gastric disorders. Numerous studies have revealed associations between *H. pylori* infection and multiple gastric diseases (14,15). *H. pylori* infection results in persistent colonization, chronic gastric inflammation and gastric atrophy (15). Patients infected with *H. pylori*, exhibiting chronic or recurrent upper abdominal symptoms, without ulceration or erosion of the gastroduodenal mucosa as identified by gastrointestinal endoscopy, are diagnosed with FD according to the Rome III criteria (3). For this reason, at least one FD subtype may be associated with the pathological state induced by *H. pylori* infection; however, to date, the data have remained inconclusive (16,17). A study demonstrated that post-infectious FD symptoms may persist following the elimination of gastrointestinal infection, in addition to the development of post-infectious irritable bowel

syndrome accompanying the colonic inflammation (18). These observations suggested that an altered inflammatory immune response may influence the development of functional gastrointestinal diseases.

Interleukin (IL)-1β and tumor necrosis factor (TNF)-α are particularly important in the initiation and amplification of the inflammatory responses to *H. pylori* infection (19,20). The two cytokines have been demonstrated to be upregulated in the *H. pylori*-infected mucosa. Furthermore, IL-1β and TNF-α are potent inhibitors of gastric acid secretion (21,22), which is a significant factor for the mediation of dyspeptic symptoms (23,24). IL-1β is a 100-fold more potent inhibitor of gastric acid secretion than proton pump inhibitors, and a 6,000-fold more potent inhibitor than H_2-receptor antagonists (H2RAs) (21).

The human IL-1β gene has multiple polymorphisms, and the C>T and T>C transitions at positions -511 and -31, respectively, have been reported to be associated with inter-individual variations in physiological IL-1β concentrations (25,26). The IL-1β -511 TT and CT genotypes are associated with increased IL-1β production compared with that of the IL-1β -511 CC genotype (25,27). The IL-1β -511 genotypes have been reported to be in linkage disequilibrium with IL-1β -31 among ethnically distinct populations (28,29).

The TNF-α gene also contains multiple polymorphisms and three of these polymorphisms, TNF-α -1031 (T>C), -863 (C>A) and -857 (C>T), which are in linkage disequilibrium, have been identified amongst Japanese individuals (30-32). These polymorphisms have been associated with increased transcriptional promoter activity (30,31).

Due to the significant role of inflammatory responses against *H. pylori* in the development of gastrointestinal diseases, multiple studies have evaluated the effects of IL-1β and TNF-α polymorphisms on the development of *H. pylori*-associated gastric diseases, including gastric cancer and peptic ulcer diseases (25,26,30). However, to the best of our knowledge, there have been no studies investigating the potential association between IL-1β and TNF-α polymorphisms, and FD. The function of IL-1β and TNF-α in the mediation of the *H. pylori*-associated inflammatory immune response and gastric acid secretion may represent a significant influencing factor in the induction of dyspeptic symptoms, and inter-individual functional differences caused by polymorphisms may be associated with altered susceptibility to the development of FD.

Accordingly, the present study was designed to evaluate a potential association between common polymorphisms in the IL-1β gene [-31 (T>C)], which is in linkage disequilibrium with IL-1β polymorphism -511 (C>T) (28,29), and TNF-α polymorphism -857 (C>T), which is in complete linkage disequilibrium with TNF-α polymorphisms -1031 (T>C) and -863 (C>A) (32), and dyspeptic symptoms. In addition, the association between these polymorphisms and various subtypes of symptoms, including the Rome III classification, were assessed.

Materials and methods

Study populations. Study populations were recruited from patients attending the Endoscopy Center of Fujita Health University Hospital (Tayoake, Japan) between January 2007 and December 2009. All patients recruited underwent upper gastroscopy for various reasons, including for a health check or a secondary complete check up of stomach cancer following a barium radiograph or complaints of abdominal discomfort. Patients diagnosed with significant upper gastrointestinal results, including peptic ulcer disease, reflex esophagitis and malignancies, identified following gastroscopy were excluded from the study. Patients with malignancies in other organs, or who had received nonsteroidal anti-inflammatory drugs, antibiotics or *H. pylori*-eradication treatment were also excluded. Patients with other diseases were also excluded via a face-to-face medical history and physical examination, including blood tests, abdominal ultrasound and electrocardiogram. Patients were required to complete a questionnaire prior to gastroscopy, and patients with dyspepsia were identified as those individuals exhibiting a primary compliant of either continuous or intermittent dyspepsia for three months, predominantly located in the upper abdomen and irrespective of the use of H2RAs or proton-pump inhibitors. Control participants were recruited from those individuals who were negative for dyspeptic symptoms within the preceding 12 months. Those who had received treatment with proton-pump inhibitory drugs or H_2-receptor antagonists during the previous 12 weeks were excluded from the control group. According to the criteria outlined, 261 subjects, comprising 126 control subjects and 135 subjects exhibiting symptoms of dyspepsia, were included in the present study. The 135 subjects with dyspeptic symptoms, were divided into the following seven categories based on their symptomatic subtypes: Upper abdominal pain (n=48), heavy feeling in stomach (n=24), upper abdominal discomfort (n=23), nausea (n=22), burning sensation (n=26), anorexia (n=6), belching (n=6) and other symptoms (n=19). Amongst the symptomatic group, 102 patients were diagnosed as having only one symptom, while the remaining 33 patients were diagnosed with 2-4 symptoms. Furthermore, dyspeptic symptoms were classified as epigastric pain syndrome (EPS)-like symptoms or postprandial distress syndrome (PDS)-like symptoms, according to the Rome III criteria (3). EPS-like symptoms were defined as epigastric pain and burning, whereas PDS-like symptoms were defined by postprandial fullness and early satiation. Dyspeptic symptoms, which met neither EPS- nor PDS-like symptoms were classified as other. According to these criteria, 71, 45 and 31 patients were diagnosed with EPS-like, PDS-like and other symptoms, respectively. Amongst these groups, 13 patients were diagnosed as having EPS- and PDS-like symptoms. *H. pylori* infection status was assessed by serological or histological analysis or a urea breath test. Patients were diagnosed with *H. pylori* infection following a positive result in at least one of these diagnostic tests. The experimental protocol was approved by the Ethics Committee of Fujita Health University School of Medicine (Toyoake, Japan) and written informed consent was obtained from all participants.

Genotyping for IL-1β and TNF-α polymorphisms. Genomic DNA was extracted from uninvolved mucosa of the gastric antrum or peripheral blood using the standard phenol/chloroform method in all patients (32,33). Subsequently, polymorphisms at position -31 (T>C) and of the IL-1β gene

Table I. Characteristics of subjects.

Variable (n)	Gender (male/female)	Age (mean ± SD)	*H. pylori* infection positive subjects n (%)
Total subjects (261)	137/124	59.9±14.1	137 (52.5)
Subjects without symptoms (126)	75/51	60.1±13.7	75 (59.5)
Subjects with dyspeptic symptoms (135)	62/73[a]	58.0±14.4	62 (45.9)[b]

[a]P=0.035 vs. subjects without symptoms; [b]P=0.035 vs. subjects without symptoms. Statistical significance was assessed using Fisher's exact test. SD, standard deviation.

Table II. Prevalence of IL-1β and TNF-α polymorphisms, in subjects with or without dyspeptic symptoms.

	IL-1β -31 genotype n (%)			TNF-α -857 genotype n (%)		
Variable n (%)	TT	TC	CC	CC	CT	TT
Total subjects (261)	51 (19.5)	127 (48.7)	83 (31.8)	192 (73.6)	54 (20.7)	15 (5.7)
Subjects without symptoms (126)	24 (19.0)	54 (42.9)	48 (38.1)	89 (70.6)	27 (21.4)	10 (7.9)
Subjects with dyspeptic symptoms (135)	27 (20.0)	73 (54.1)	35 (25.9)	103 (76.3)	27 (20.0)	5 (3.7)

IL-1β -31: Subjects without symptoms vs. subjects with dyspeptic symptoms, CC vs. TT+TC, P=0.046; OR (95% CI) for CC=0.57 (0.34-0.96); OR (95% CI) for TT+TC=1.76 (1.04-2.98). Statistical significance was assessed using the two-sided Fisher's exact test. IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; OR, odds ratio; CI, confidence interval.

and -857 (C>T) of the TNF-α gene were genotyped using the polymerase chain reaction-restriction fragment length polymorphism method (32,33).

Statistical analysis. Variations in genotype frequencies between two groups were determined using one- or two-sided Fisher's exact test. Furthermore, the strength of association between genotype frequencies and the disease was assessed by calculating the odds ratio (OR) and 95% confidence intervals (CIs). P<0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of the study population. The characteristics of the study population are summarized in Table I. In the comparison between subjects without symptoms and subjects with dyspeptic symptoms, the mean age was not significantly different between the two groups; however, symptoms of dyspepsia were observed to be significantly greater in female subjects and subjects positive for *H. pylori*-infection.

IL-1β polymorphisms are associated with the risk of developing dyspeptic symptoms. The prevalence of IL-1β and TNF-α polymorphisms amongst all subjects, and in subjects with or without dyspeptic symptoms are exhibited in Table II. All polymorphisms were successfully genotyped and the prevalence of the polymorphisms detected was similar to that reported by previous Japanese studies (32,33).

A comparison of genotype frequencies revealed that the frequency of the IL-1β -31 CC genotype was significantly lower among subjects with dyspeptic symptoms when compared with subjects without symptoms (38.1 vs. 25.9%, P=0.046). The ORs and 95% CIs of the CC genotype were, 0.57 and 0.34-0.96. Conversely, the other genotypes investigated (TT and TC) were associated with a significantly higher risk of dyspeptic symptoms (OR, 1.76; 95% CI, 1.04-2.98).

No significant associations were identified between the TNF-α -857 polymorphisms and dyspeptic symptoms.

IL-1β polymorphisms are associated with various subtypes of symptoms. The prevalence of IL-1β and TNF-α polymorphisms in seven distinct symptomatic subtypes are shown in Table III. The frequency of the IL-1β -31 CC genotype was found to be significantly lower amongst subjects with upper abdominal pain when compared with subjects without symptoms (P=0.003). The OR and 95% CI of the IL-1β -31 CC genotype were 0.28 and 0.12-0.67. However, the other genotypes investigated (TT and TC) were associated with a significantly higher risk of upper abdominal pain (OR, 3.60; 95% CI, 1.50-8.68) than that of the CC. It was also demonstrated that the frequency of the IL-1β -31 CC genotype was lower in subjects with nausea and burning sensations, but this association was not significant (P=0.09 and 0.07, respectively). The prevalence of IL-1β and TNF-α polymorphisms, and distinct symptomatic subtypes according to the Rome III criteria are shown in Table IV. It was revealed that the frequency of the IL-1β -31CC genotype was significantly lower amongst subjects with EPS-like symptoms (P=0.003). The OR and 95% CI of the CC genotype were 0.14 and 0.07-0.28. Conversely, the other genotypes investigated (TT and TC) were associated with a significantly higher risk

Table III. Prevalence of IL-1β and TNF-α polymorphisms, in various symtoms subtypes.

	IL-1β -31 genotype n (%)			TNF-α -857 genotype n (%)		
Symptom n (%)	TT	TC	CC	CC	CT	TT
Subjects without symptoms (126)	24 (19.0)	54 (42.9)	48 (38.1)	89 (70.6)	27 (21.4)	10 (7.9)
Upper abdominal pain (48)	12 (25.0)	29 (60.4)	7 (14.6)	32 (66.7)	12 (25.0)	4 (8.3)
Heavy feeling in stomach (24)	3 (12.5)	15 (62.5)	6 (25.0)	20 (83.3)	4 (16.7)	0 (0)
Upper abdominal discomfort (23)	6 (26.1)	8 (34.8)	9 (39.1)	20 (87.0)	2 (8.7)	1 (4.3)
Nausea (22)	7 (31.8)	11 (50.0)	4 (18.2)	17 (77.3)	5 (22.7)	0 (0)
Burning sensation (26)	7 (26.9)	14 (53.9)	5 (19.2)	18 (69.2)	7 (26.9)	1 (3.9)
Anorexia (6)	1 (17.7)	2 (33.3)	3 (50.0)	5 (83.3)	1 (17.7)	0 (0)
Belching (6)	1 (17.7)	2 (33.3)	3 (50.0)	5 (83.3)	1 (17.7)	0 (0)
Other (19)	2 (10.5)	11 (57.9)	6 (31.6)	15 (78.9)	4 (21.1)	0 (0)

In total, 102 patients were diagnosed as having a single symptom, while the remaining 33 patients were diagnosed as having 2-4 symptoms. IL-1β -31: Subjects without symptoms vs. upper abdominal pain, CC vs. TT+TC, P=0.003; OR (95% CI) for CC=0.28 (0.12-0.67); OR (95% CI) for TT+TC=3.60 (1.50-8.68). Subjects without symptoms vs. nausea, CC vs. TT+TC, P=0.09. Subjects without symptoms vs. burning sensation, CC vs. TT+TC, P=0.07. Statistical significance was assessed using the two-sided Fisher's exact test. IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; OR, odds ratio; CI, confidence interval.

Table IV. Prevalence of IL-1β and TNF-α polymorphisms, in symptoms subtypes according to Rome III.

	IL-1β -31 genotype n (%)			TNF-α -857 genotype n (%)		
Variable n (%)	TT	TC	CC	CC	CT	TT
Subjects without symptoms (126)	24 (19.0)	54 (42.9)	48 (38.1)	89 (70.6)	27 (21.4)	10 (7.9)
EPS-like symptoms (71)	16 (22.5)	42 (59.2)	13 (18.3)	51 (71.8)	16 (22.6)	4 (5.6)
PDS-like symptoms (45)	6 (13.3)	25 (55.6)	14 (31.1)	37 (82.2)	8 (17.8)	0 (0)
Other (32)	6 (18.7)	15 (46.9)	11 (34.4)	24 (75.0)	7 (21.9)	1 (3.1)

Thirteen patients were diagnosed as having EPS- and PDS-like symptoms. IL-1β -31: Subjects without symptoms vs. EPS-like symptoms, CC vs. TT+TC, P=0.003; OR (95% CI) for CC=0.14 (0.07-0.28); OR (95% CI) for TT+TC=7.25 (3.60-14.61). Statistical significance was determined using the two-sided Fisher's exact test.

of upper abdominal pain (OR, 7.25; 95% CI, 3.60-14.61) compared with that of the CC genotype.

Logistic regression analysis. As a significant correlation between female gender and positive *H. pylori* infection and symptoms of dyspepsia, logistic regression analysis with adjustments for these factors was performed in order to verify the associations between the IL-1β -31 genotype and susceptibility to dyspeptic symptoms, including upper abdominal pain and EPS-like symptoms. It was revealed that the association between IL-1β -31 genotype and all dyspeptic symptoms did not remain following logistic regression analysis (P=0.078; adjusted OR and 95% CI for CC: 0.62 and 0.36-1.06; adjusted OR and 95% CI for TT+TC: 1.62, and 0.95-2.98).

However, the associations between IL-1β -31 genotype and upper abdominal pain and EPS-like symptoms remained following logistic regression analysis (upper abdominal pain: P=0.014, adjusted OR and 95% CI for CC were 0.34 and 0.14-0.80, adjusted OR and 95% CI for TT+TC were 2.94, and 1.25-6.94; and EPS-like symptoms: P=0.015, adjusted OR and 95% CI for CC were 0.41 and 0.20-0.84, adjusted OR and 95% CI for TT+TC were 2.44 and 1.19-4.98).

Discussion

The results of the present study indicated that the IL-1β -31 polymorphism was associated with susceptibility to dyspeptic symptoms; in particular, upper abdominal pain and EPS-like symptoms. The CC genotype homozygous variant was associated with a significantly lower risk of all dyspeptic symptoms, abdominal pain and EPS-like symptoms. The associations with upper abdominal pain and EPS-like symptoms were also retained as an independent factor following logistic regression analysis. Conversely, the other genotypes investigated (TT and TC) were associated with a significantly higher risk of these symptoms.

The IL-1β -31 C allele had previously been reported to be in linkage disequilibrium with the IL-1β -511 T allele in ethnically diverse populations (28,29). The IL-1β -511 TT and

CT variant genotypes have also been reported to be associated with increased IL-1β production in comparison to that of the IL-1β -511 CC genotype (25,26). Wang *et al* (19) found that enhanced IL-1 gene expression was associated with more severe corpus gastritis and suppressed gastric acid secretion. Their group also reported that the IL-1β -511 variant allele, a high IL-1β-producing genotype, was associated with lower gastric acid secretion and atrophic gastritis in *H. pylori*-infected subjects (33). Therefore, increased production of IL-1β by high-producing genotypes, resulted in enhanced suppression of gastric acid secretion (25,26). It has also been demonstrated that direct acid infusion into the stomach or duodenum affects gastroduodenal motility, as well as sensation, and a role for gastric acid in FD has been suggested (23,24). In this context, it may be reasonable to hypothesize that the IL-1β -31 CC genotype, a high-producing genotype, may be associated with enhanced suppression of gastric acid secretion and therefore a reduction in the risk of developing dyspeptic symptoms. In addition, since IL-1β is important in initiating and amplifying inflammatory responses in the stomach, particularly following *H. pylori* infection, inter-individual variations in the inflammatory immune response generated by IL-1β -31 polymorphisms may also explain the results observed in the present study.

Notably, no significant associations were detected between TNF-α -857 polymorphisms and dyspeptic symptoms, including the various subtypes, which suggested that the TNF-α -857 polymorphism was unlikely to be an influencing factor in the development of dyspeptic symptoms.

Amongst the symptomatic subtypes, the IL-1β -31 polymorphism was closely associated with upper abdominal pain and EPS-like symptoms, which suggested that symptomatic subgroups of FD may have distinct genetic backgrounds. FD is varied in its pathophysiology, clinical progression, prognosis and response to treatment; thus, it was hypothesized that FD may be a syndrome in which multiple pathogenic mechanisms converge to generate diverse clinical phenotypes. A greater emphasis on disease heterogeneity may be required in order to improve the diagnosis and treatment of FD. The results of the present study suggested that the IL-1β -31 polymorphism may be an influencing factor for specific subgroups of FD, including upper abdominal pain and EPS-like symptoms.

To the best of our knowledge, the present study was the first to investigate the potential association between IL-1β -31 polymorphisms and symptoms of dyspepsia. However, there were certain limitations to this preliminary study. In the present study, all participants were recruited from subjects who were undergoing upper gastroscopy. Therefore, ulcer disease, reflex esophagitis and malignancies that may be associated with abdominal symptoms were able to be excluded. However, the patient cohort comprised a majority of subjects of greater age than that of the general population of patients with FD (34), who were undergoing upper gastroscopy for a health check or a secondary complete check up of stomach cancer following barium radiography.

In addition, the dyspeptic subjects recruited had a primary complaint of either continuous or intermittent dyspepsia for three months. However, the Rome III classification could not be completely applied in a significant percentage of subjects as the onset of symptoms occurred <6 months prior to diagnosis.

Furthermore, the association between IL-1β -31 polymorphisms, and upper abdominal pain and EPS-like symptoms was obtained from a relatively small sample size in the subgroup analysis, and, it should be noted that the effect of type II errors cannot be excluded in such a small sample size. Therefore, a replication study, using larger samples, including ethnically diverse populations, which may be more representative of patients with FD is required.

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