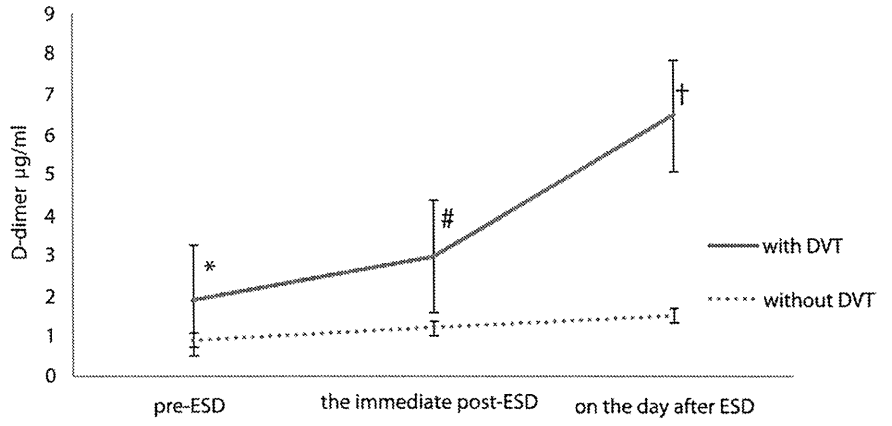

Figure 1. Differences in perioperative D-dimer levels in patients undergoing ESD. After interactions for times and groups of D-dimer levels had been statistically confirmed, D-dimer levels were compared between patients with versus without DVT at each time point. D-dimer levels were higher in patients with than without DVT at all time points examined. *DVT*, deep vein thrombosis; *ESD*, endoscopic submucosal dissection.

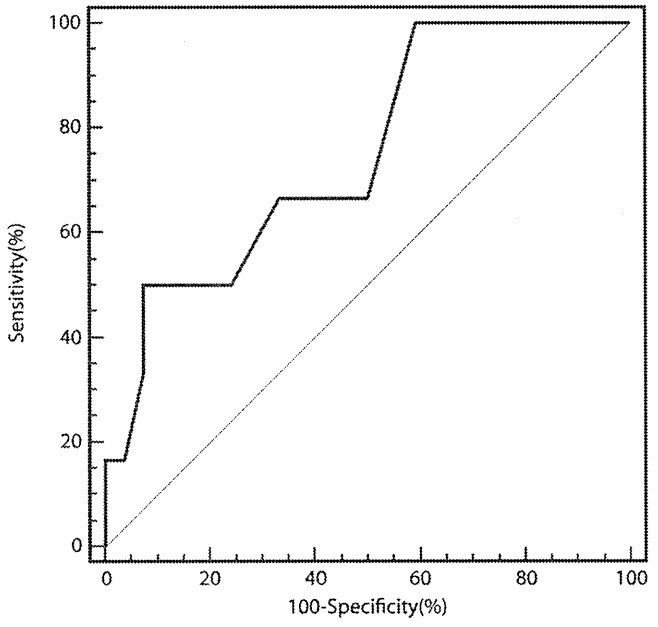

Figure 2. Receiver operating characteristic curve of D-dimer levels before endoscopic submucosal dissection. The curves are plotted with sensitivity (*y value*) and |100 − specificity| (*x value*).

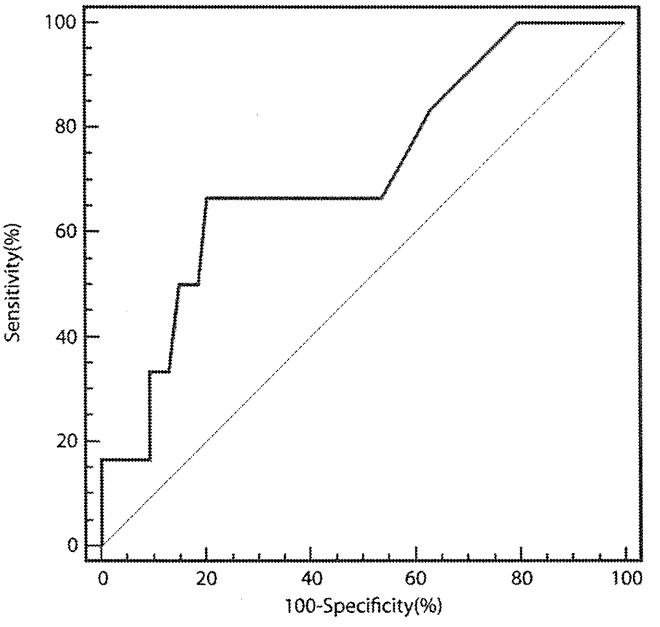

Figure 3. Receiver operating characteristic curve of D-dimer levels measured immediately after endoscopic submucosal dissection. The curves are plotted with sensitivity (*y value*) and |100 − specificity| (*x value*).

chosen by using the distance to the ROC curve and the Youden index for the D-dimer level the day after ESD. The resulting cut-off point was 1.9 μg/mL (sensitivity 83.33%; specificity 79.63%) for patients who underwent ESD (Table 1).

Factors in association with DVT development identified through univariate analyses

Based on the ROC curve analysis results and optimal cut-off points of D-dimer levels determined earlier, a D-dimer level above 1.9 μg/mL the day after ESD was used in univariate analyses.

As shown in Table 2, univariate analysis demonstrated that a high D-dimer level the day after ESD (P = .0009; 95% CI, 1.264-48.357; OR, 7.818) and comorbidities (P = .0267; 95% CI, 1.450-58.367; OR, 9.200) were significantly associated with the development of DVT. However, no other risk factors examined in the present study were significantly associated with DVT development.

Warfarin therapy was started for 6 patients with DVT 3 days after ESD, and disappearance of the thrombus was confirmed by ultrasonography 3 to 6 months after ESD in all patients.

DISCUSSION

This is the first prospective study to assess the incidence of DVT after ESD for gastric neoplasia. The overall fre-

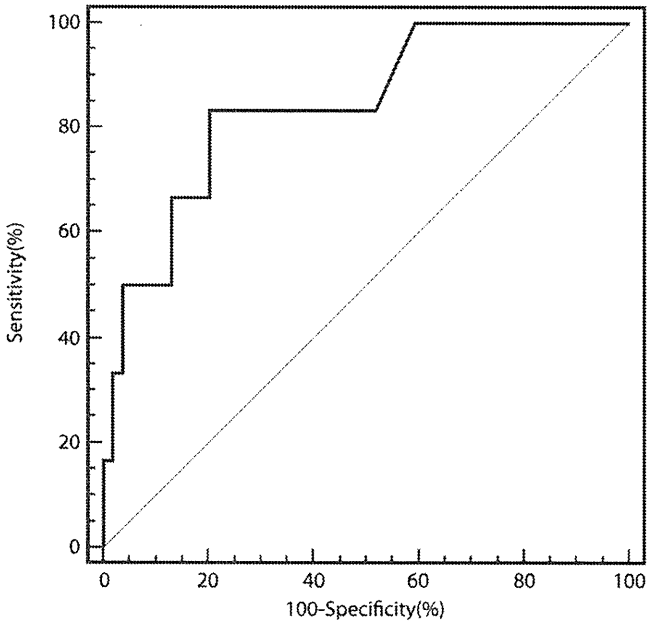

Figure 4. Receiver operating characteristic curve of D-dimer levels measured the day after endoscopic submucosal dissection. The curves are plotted with sensitivity (*y value*) and [100 − specificity] (*x value*).

quency of asymptomatic DVT after ESD was 10.0%. The incidence of objectively confirmed, hospital-acquired VTE is approximately 40% to 60% after major orthopedic surgery.[1] The overall incidence of VTE also has been shown to be approximately 20% for abdominal surgery without appropriate thromboprophylaxis.[14] The risk of thromboembolism with an ESD procedure was shown to be moderate, in this study, in accordance with American College of Chest Physicians guidelines.[1] For patients who are at moderate risk for VTE and also have a high risk of bleeding, the guidelines recommend using mechanical thromboprophylaxis (intermittent pneumatic compression, venous foot pump, and/or graduated compression stockings).

The American Society of Clinical Oncology VTE guideline panel has recommended that all hospitalized patients with cancer be considered for VTE prophylaxis with anticoagulants in the absence of bleeding or other contraindications. The following treatment-related factors were identified as VTE risk factors in patients with malignant disease: recent major surgery, current hospitalization, active chemotherapy, active hormonal therapy, current or recent antiangiogenic therapy, current erythropoiesis-stimulating agent use, and the presence of central venous catheters. However, endoscopic therapies have not yet been demonstrated to be treatment-related risk factors for VTE. Although ESD treatment is not considered to be more invasive than abdominal surgery, the risk for thromboembolism (DVT) with ESD was moderate, that is, almost the same level as that of abdominal surgery. One explanation for these findings may be that ESD procedures often require patients to remain in the same position for prolonged periods with the use of intravenous sedation. In addition, the increased intraabdominal pressure created by air insufflations during ESD may cause venous pooling in the legs, via vessel wall damage, as suggested by animal studies of pneumoperitoneum.[15] Therefore, it may be more appropriate for air insufflations during ESD to use rapidly absorbed CO_2 gas in order to prevent gas accumulation in the intestine.

A D-dimer level exceeding 1.9 μg/mL the day after ESD was thought to be the most reliable marker associated with DVT development. D-dimer levels, especially the day after ESD, appear to have specific features for DVT development in patients undergoing ESD. The D-dimer is a marker of endogenous fibrinolysis and should therefore be detectable in patients with VTE.[16] The D-dimer assay has generally been reported to be a sensitive but nonspecific marker of DVT, thus making it a good rule-out test with appropriate pretest probability.[17,18] D-dimer levels usually show large individual deviations and could be elevated by bleeding and coagulation during the ESD procedure. Although we could not estimate precise amounts of bleeding during ESD procedures in this study, individual deviations in D-dimer levels could be corrected by the analysis procedure used in the present study. Furthermore, no patients needed blood transfusions in this study. In our experience, bleeding that requires blood transfusion appears to affect D-dimer levels. Although it is difficult to estimate precise amounts of bleeding during ESD procedures, in cases requiring transfusion, the volume of blood transfused might have to be assessed to determine the impact of bleeding during ESD procedures on D-dimer levels. In patients undergoing orthopedic or general surgery, D-dimer levels the day after surgery varied widely because of differences in internal hypercoagulability and surgical procedures, such as the amount of bleeding and frequencies of transient coagulation. Despite this variability, Dindo et al[19] reported that D-dimer levels do not increase after superficial surgery (no opening of the abdominal cavity, such as open hernia repairs), although D-dimer levels after general abdominal surgery are elevated even in patients without DVT. On the other hand, in our study of patients undergoing ESD, which does not involve opening the abdominal cavity, there were no significant changes in D-dimer levels at any of the time points examined in patients without DVT. However, D-dimer levels after ESD in patients with DVT were significantly elevated and were higher than in those patients without DVT. D-dimer levels are more specifically related to DVT after ESD. This is different from D-dimer levels after general abdominal surgeries, which are elevated even in patients without DVT.

Univariate analysis demonstrated that a high D-dimer level the day after ESD and comorbidities were significantly associated with the development of DVT, but other predictors were not. Because DVT development is complicated by some factors, multivariate analysis is desirable to evaluate predictors for DVT development after ESD.

TABLE 1. Cut-off points of D-dimer levels the day after ESD, showing optimal performance for diagnostic accuracy of DVT development

D-dimer levels (μg/mL)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Likelihood ratio	Distance to ROC curve*	Youden index†
≥0.4	100	0	10		1	1	0
>0.8	100	40.74	15.8	100	1.69	0.5926	0.4074
>0.9	83.33	48.15	15.2	96.3	1.61	0.5446	0.3148
>1.9	83.33	79.63	31.2	97.7	4.09	0.2632	0.6296
>2	66.67	79.63	26.7	95.6	3.27	0.3906	0.4630
>2.2	66.67	87.04	36.4	95.9	5.14	0.3576	0.5371
>2.4	50	87.04	30	94	3.86	0.5165	0.3704
>4.4	50	96.3	60	94.5	13.5	0.5013	0.4630
>4.6	33.33	96.3	50	92.9	9	0.6677	0.2963
>4.7	33.33	98.15	66.7	93	18	0.6670	0.3148
>5.6	16.67	98.15	50	91.4	9	0.8335	0.1482
>7.9	16.67	100	100	91.5		0.8333	0.1667
>23.3	0	100	90			1	0

ESD, Endoscopic submucosal dissection; *DVT,* deep vein thrombosis; *PPV,* positive predictive value; *NPV,* negative predictive value; *ROC,* receiver operating characteristic.
*Distance to ROC curve = $\surd[(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2]$.
†Youden index = sensitivity + specificity − 1.

However, in this study, multivariate analysis could not be done with such a small number of outcomes to assess. Further studies are necessary to permit meaningful multivariate analysis.

Interestingly, statistically significant differences in D-dimer levels between patients with DVT and without DVT were demonstrated not only immediately after ESD and the day after ESD but also before ESD. Mukubo and Kawamata[10] reported that patients with rheumatoid arthritis, a typical chronic inflammatory disease, have higher D-dimer levels preoperatively than patients with non-inflammatory osteoarthrosis, and that the D-dimer level was still elevated 1 week after the operation. This observation means that distinct disorders could show different perioperative hemostatic changes. These findings indicate that intrinsic or comorbid factors might be involved in DVT development after ESD in patients with gastric neoplasms.

The incidence of VTE had been considered to be lower in Japan than in Western countries. However, the mortality rate from PTE is rapidly increasing in Japan according to reports of routine autopsy examinations and has become comparable to that in Western countries, because this disease is now more often correctly diagnosed, Japanese lifestyles have become westernized, and the BMI has increased.[1,20] We showed that the overall frequency of DVT after ESD was 10.0%. However, all 6 patients with DVT were asymptomatic, and if ultrasonography of the lower limbs had not been performed, we would not have detected the DVT. In addition, the occurrence of PTE, a clinically serious condition, is thought to be rare after ESD because early ambulation is usually allowed, unlike orthopedic surgeries, in our setting. Nevertheless, when prolonged bed rest is required because of special situations such as ESD complications (perforation or late bleeding), a serious condition such as PTE might, on rare occasions, develop. Our results raise the possibility that greater awareness of the need for VTE prophylaxis is required.[21]

In conclusion, patients undergoing ESD were considered to be at moderate risk, as defined by the American College of Chest Physicians guidelines, for thromboembolism. D-dimer levels, especially on the day after ESD, appear to have specific features associated with DVT development in patients undergoing ESD. Because of the high incidence of DVT after ESD procedures, mechanical thromboprophylaxis should be considered in these patients, except for those with comorbidities, such as arterial circulatory deficits, cellulitis or thrombophlebitis of the lower limbs, congestive heart failure, and acute myocardial infarction, which might be aggravated by thromboprophylaxis. However, mechanical thromboprophylaxis has yet to be assessed by an interventional, randomized trial. Thus, further study is needed to determine appropriate

TABLE 2. Factors in association with DVT development identified through univariate analyses

Characteristic	Univariate analysis DVT, no. (%) Absence	Presence	*P* value
Sex			.7680
Female	14 (25.9)	2 (33.3)	
Male	40 (74.1)	4 (66.7)	
Age, y			.2212
<65	11 (20.4)	0 (0)	
≥65	43 (79.6)	6 (100)	
BMI (kg/m^2)			.6840
<25	41 (75.9)	5 (83.3)	
≥25	13 (24.1)	1 (16.7)	
Anti-thrombotic drugs			.9114
Not used	44 (81.5)	5 (83.3)	
Used	10 (18.5)	1 (16.7)	
Warfarin	1 (1.85	1 (16.7	.0551
Aspirin	6 (11.1)	0 (0)	.3894
Ticlopidine	1 (1.85)	0 (0)	.7368
Ethyl icosapentate	1 (1.85)	0 (0)	.7368
Dipyridamole	1 (1.85)	0 (0)	.7368
Comorbidity			.0267
Absence	49 (90.7)	3 (50.0)	
Presence	5 (9.3)	3 (50.0)	
Stroke	2 (3.70)	0 (0)	.6316
Heart failure	2 (3.70)	1 (16.7)	.1669
Renal failure	0 (0)	2 (33.3)	.0001
COPD	1 (1.85)	0 (0)	.7368
Operative time, min			.2839
<100	21 (38.9)	1 (16.7)	
≥100	33 (61.1)	5 (83.3)	
D-dimer level the day after ESD (μg/mL)			.0009
≤1.9	43 (79.6)	1 (16.7)	
>1.9	11 (20.4)	5 (83.3)	

DVT, Deep vein thrombosis; *BMI*, body mass index; *COPD*, chronic obstructive pulmonary disease; *ESD*, endoscopic submucosal dissection.

thromboprophylaxis recommendations for patients undergoing ESD.

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—Review—

Pathophysiology of Functional Dyspepsia

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Abstract

Functional dyspepsia is a highly prevalent and heterogeneous disorder. Functional dyspepsia involves many pathogenic factors, such as gastric motility disorders, visceral hypersensitivity, psychological factors, *Helicobacter pylori* infection, and excessive gastric acid secretion. The present article provides an overview of pathogenetic factors and pathophysiologic mechanisms.
(J Nippon Med Sch 2011; 78: 280–285)

Key words: functional dyspepsia, gastric emptying, ghrelin, post-infectious functional dyspepsia

Introduction

Functional dyspepsia (FD) is divided into two subgroups according to the Rome III criteria: epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS)[1]. Most patients with FD complain of symptoms related to the intake of meals; however, the pathophysiology of FD remains poorly understood[2-4]. A number of potentially important abnormalities have been reported in FD patients, including impaired fundic accommodation[5], gastric hypersensitivity to distention[6], abnormal duodenojejunal motility[7], duodenal motor and sensory dysfunction[8], duodenal hypersensitivity[9] and *Helicobacter pylori* infection[10]. Although the Rome III criteria exclude gastroesophageal reflux symptoms from the symptoms of FD patients, some degree of overlap between the symptoms of non-erosive reflux disease (NERD) and FD is inevitable. In addition, the symptoms of both FD and NERD can include impaired gastric motility. Most patients with FD and NERD complain of several symptoms related to meals, however the pathophysiology of these diseases remains poorly defined[2,3]. Forty to sixty percent of patients with FD also have *H. pylori* gastritis[11,12], but whether *H. pylori* is the cause of the symptoms associated with FD is unclear[13,14]. Impaired gastric motility is strongly associated with gastric emptying and gastric accommodation and has been implicated in the pathophysiology of functional dyspepsia, a common gastrointestinal disorder[15]. Delayed gastric emptying is reportedly present in 25% to 50% of patients with FD[16].

FD and Gastric Emptying

The standard method for measuring gastric

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emptying is the radioactive isotope method[17]. The ^{13}C-acetate breath test is a reliable and non-invasive tool for analyzing gastric emptying rates without radiation exposure and is comparable to scintigraphy[18]. Most studies following the Rome II criteria have failed to find a good correlation between clinical symptoms and gastric emptying[19]. However, Sarnelli et al. have reported that female sex, postprandial fullness and vomiting are strong independent predictors of slow gastric emptying[20]. Three large-scale single-center studies from Europe have shown that patients with delayed gastric emptying for solids are more likely to report postprandial fullness, nausea and vomiting[21,22], although two other large multi-center studies in the United States found no association or found only a weak association[23,24]. Because the most important characteristic symptom in patients with PDS patients is postprandial fullness, a clearer understanding of the mechanics of gastric emptying would help elucidate the etiology of PDS.

Patients with NERD and FD

Several mechanisms have been proposed for the pathogenesis of NERD patients, including visceral hypersensitivity, prolonged contraction of the esophagus and psychological factors[25-27]. Previous studies have correlated FD and NERD symptoms with psychological distress[28,29]. In our study, scores of the Self-Rating Questionnaire for Depression (SRQ-D) reflecting distress and a state of depression were significantly higher in patients with FD and NERD than in healthy volunteers[30]. Quigley et al. have reported that the precise prevalence of delayed gastric emptying remains to be defined in gastroesophageal reflux disease[31]. Our data show that 33% of patients with NERD exhibit delayed gastric emptying. Therefore, administration of mosapride citrate in addition to omeprazole decreased gastro-esophageal reflux and improved gastric emptying in patients with proton pump inhibitor-resistant NERD and delayed gastric emptying[32]. Uemura et al. have reported that the CYP2C19 genotype has no significant effect on the rate of complete resolution of heartburn in patients with NERD[33].

Ghrelin and FD Patients

Ghrelin is a 28-amino acid peptide produced in the stomach. It is an endogenous ligand for the growth hormone secretagogue receptor (GHSR) in the oxyntic gland of the stomach[34]. In rodents, central or peripheral administration of ghrelin stimulates gastric contraction and emptying[15] and shows prokinetic effects in a postoperative ileus model in rats[35]. In human studies, ghrelin infusion also increases food intake and the sensation of hunger compared with saline infusion alone. These widespread physiological functions have encouraged research to assess the efficacy of exogenous ghrelin administration as a novel therapy for various disorders such as growth hormone (GH) deficiency, cachexia, anorexia nervosa, gastroparesis, functional dyspepsia and cancer anorexia. Administration of physiological doses of exogenous ghrelin to humans dose not significantly alter gastric motility[36,37]. However intravenous administration of high doses (40 μg) of ghrelin in healthy volunteers induces a premature gastric phase III of the migrating motor complex and increases proximal stomach tone[38]. Thus, several studies have shown that infusion of ghrelin as opposed to placebo accelerates gastric emptying and decreases meal-related symptoms in patients with gastroparesis[39-41]. In addition, repeated intravenous infusions of ghrelin (3 μg/kg) twice a day to five patients with functional dyspepsia for 2 weeks increased daily food intake by approximately 30% compared with levels before and after ghrelin treatment[42]. We also believe that impaired gastric emptying is reflected by low levels of acylated ghrelin in patients with PDS[30]. Suzuki et al. have reported that plasma ghrelin levels correlate well with the serum pepsinogen I/II (PGI/II) ratio[43] and decrease as gastric mucosal atrophy worsens. Therefore, we have to consider that the degree of advance of gastric atrophy is negatively correlated with serum levels of ghrelin. Acylated ghrelin has been shown to accelerate gastric emptying, increase gastric tone, and induce premature interdigestive migrating motor complex activity[44,45]. In contrast,

desacylated-ghrelin has been reported to inhibit gastric emptying without altering small intestinal transit[16,17]. We have previously reported that there was a significant relationship between low levels of acylated ghrelin linked to appetite and Tmax value[30]. However, we have found that the score for feeling of hunger is not significantly (p=0.473) associated with acylated-ghrelin levels[18]. Takeda et al. have reported that the cisplatin-induced decreases in the plasma acylated-ghrelin level and food intake are mediated by 5-HT2B/2C receptors and suppressed by flavonoids in Rikkunshito[19].

H. pylori Infection and FD

Several studies and meta-analysis have tried to establish a relationship between *H. pylori* infection and FD. The relationship between *H. pylori* infection and FD patients is still controversial. McColl et al. have reported that *H. pylori* eradication therapy is effective for resolving symptoms in patients with FD[11]. On the other hand, Blum et al. have reported that in patients with FD, the eradication of *H. pylori* is not likely to relieve symptoms[50]. A recently published meta-analysis suggests that *H. pylori* eradication at 12 months has a small but statistically significant beneficial effect on symptoms in FD[51]. The main reason for *H. pylori* eradication in patients with FD may be related more to other potential beneficial effects than to symptomatic improvement[52].

Post-infectious FD

Post-infectious FD has first been proposed as a possible clinical entity based on a large retrospective, tertiary referral center study, which showed that a subset of dyspeptic patients has a history suggestive of post-infectious dyspepsia[53]. Indeed, in a population with an outbreak of salmonella gastroenteritis, the prevalence of FD was significantly increased up to 1 year after the acute event[54]. Tack et al. have reported that 25% of the patients with FD report an acute onset and that 17% of FD patients report an acute onset accompanied by signs suggestive of an acute gastrointestinal infection. These findings suggest that FD, similar to other functional bowel disorders, such as irritable bowel syndrome (IBS) and gastroparesis, may occur following an acute intestinal infection[28]. Spiller et al. have reported that numbers of mucosal T cells are increased in patients with post-infectious IBS[55]. In contrast, in our study, duodenal Vδ1 T cells and CD3-positive T cell counts did not differ among patients with post-infectious FD, EPS, or PDS and healthy volunteers[56]. In addition, Spiller et al. have also reported that numbers of enteroendocrine cells are increased in post-infectious IBS patients[55]. 5-HT is thought to be linked to the regulation of secretion, motility and sensory events. However, in the present study, there was no significant difference in numbers of serotonin producing cells of the duodenum amongst the study groups (EPS, PDS, post-infectious FD and healthy volunteers). Considering our results, we speculate that the duodenitis of post-infectious FD patients may depend on the accumulation of macrophages and eosinophils and an accompanying partial loss of villi[56]. Talley et al. and Toukan et al. have reported that there is a significant association of the number of migrated eosinophils in the duodenum in subjects with non-ulcer dyspepsia[57,58]. We have reported that numbers of eosinophils and of CCR2-positive macrophages in post-infectious FD are significantly higher than those in healthy volunteers. CCR2 expression level is regulated by monocyte chemoattractant protein-1 (MCP-1)-stimulated prostaglandin E2 production[59,60]. Kindt et al. have also reported that macrophage accumulation in the duodenum is increased in post-infectious FD patients[61]. In turn, these mediators which migrate inflammatory cells such as macrophages and eosinophils may cause sensory-motor dysfunction and produce such clinical symptoms as epigastric burning.

Duodenal Sensitivity to Lipids or Acid

In both healthy subjects and in patients with FD, duodenal perfusion with nutrient lipids, but not with glucose, enhances the perception of gastric distention[62]. These effects of duodenal lipid infusion

require lipid digestion and the subsequent release of a cholecystokinin A receptor antagonist[63]. Based on these observations, it has been proposed that increased sensitivity to duodenal lipids infusion may be a relevant pathophysiologic mechanism in FD[61].

Conclusions

The pathophysiology of FD involves many factors such as gastric motility, hypersensitivity, psychological factors and genetics. These factors interactively contribute to the manifestation of FD symptoms. Understanding of the underlying pathogenetic mechanisms might lead to better targeting of treatment in these patients with FD.

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(Received, July 21, 2011)
(Accepted, August 25, 2011)

Neurogastroenterology & Motility

Neurogastroenterol Motil (2011) **23**, 1073–e535 doi: 10.1111/j.1365-2982.2011.01781.x

G-protein β3 subunit 825CC genotype is associated with postprandial distress syndrome with impaired gastric emptying and with the feeling of hunger in Japanese

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Abstract

Background *G-protein dysfunction related alteration of intracellular signal transduction might be linked to various abnormalities of functional gastrointestinal (GI) disorders. Serotonin (5-hydroxytryptamine; 5-HT) as well as G-protein is also key signaling molecule sensorimotor functions in the GI tract. Thus, this study aims to evaluate the correlation between gastric emptying and GNβ3 and 5-HTs polymorphisms in functional dyspepsia (FD) as defined by Rome III classification.* ***Methods*** *Seventy-four patients presenting with typical symptoms of FD (epigastric pain syndrome: EPS, n = 24; postprandial distress syndrome: PDS, n = 51) and sixty-four healthy volunteers were enrolled. Gastric motility was evaluated with the T_{max} value using the ^{13}C-acetate breath test. We used Rome III criteria to evaluate upper abdominal symptoms and SRQ-D scores to determine depression status. GNβ3-C825T, 5-HT_{1A}-C1019G, 5-HT_{2A}-G1438A, 5-HT_{3A}-C42T, and 5-HT_{4A}-G353 + 6A polymorphisms were analyzed in DNA from blood samples of enrolled subjects. Genotyping was performed by polymerase chain reaction.* ***Key Results*** *There was a significant relationship (P = 0.045) between GNβ3 825CC genotype and PDS patients without gastro-esophageal reflux symptoms with impaired gastric emptying. In Japanese, GNβ3 825CC genotype in FD patients was significantly associated (P = 0.0485) with the feeling of hunger compared with GNβ3 825CT and TT genotypes.* ***Conclusions & Inferences*** *Our results suggest that the GNβ3 825CC genotype is significantly associated with PDS patients without gastro-esophageal reflux with impairments of gastric emptying and also with the feeling of hunger in patients with FD. Further studies are needed to clarify whether the GNβ3 825CC genotype is linked to disturbances of gastric emptying via altered signal transduction responses.*

Keywords *functional dyspepsia, gastric motility, GNβ3, polymorphism, postprandial distress syndrome.*

INTRODUCTION

Recently, Functional dyspepsia (FD) has been subclassified into two new disease categories under the Rome III classification, epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS).[1] Although Rome III criteria exclude gastro-esophageal reflux symptoms from the clinical symptoms of FD patients, some degree of overlap between the symptoms of non-erosive reflux disease (NERD) and FD is inevitable. Impairment of gastric motility such as gastric emptying is strongly associated with the pathophysiology of FD, one of the most common gastrointestinal (GI) disorders.[2] Disturbances of physiological gastric emptying occur with a variety of symptoms ranging from premature saturation, fullness, nausea, vomiting, epigastric pain, and acid reflux in patients with delayed emptying in FD patients. We have previously reported that T_{max} value as a marker of gastric emptying in PDS patients was significantly greater compared with that of healthy volunteers.[3] We have reported that prokinetics such as mosapride citrate improve clinical symptoms through affecting T_{max} value in proton pump inhibitor (PPI)-resistant NERD patients with

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Received: 23 May 2011
Accepted for publication: 20 July 2011

impaired gastric emptying.[4] These results suggest that T_{max} value is one of the useful marker for considering the management of FD and NERD patients.

G-protein is composed of different α, β, and γ subunit isoforms, the $\beta\gamma$ subunit forming a functional monomer. On receptor activation, both α and $\beta\gamma$ subunits dissociate from the receptor and in turn modulate a large variety of intracellular effector system. Accordingly, G-protein dysfunction potentially could block intracellular signal transduction. The GNβ3 gene encodes the Gβ3 subunit of heterotrimeric G proteins, which are key components of intracellular signal transduction that are widely present in cells of the body.[5] Thus, G-protein dysfunction related alteration of intracellular signal transduction might be linked to various abnormalities of functional GI disorders including disturbed gut sensory or motor function,[6–8] dysfunction of the autonomic nervous system,[9] and underlying psychiatric disturbances.[10] A common C825T polymorphism has been described in the gene GNβ3 that encodes the β3 subunit of heterotrimeric G-proteins. Homozygous 825C allele carriers (CC genotype) form only minute amounts of the β3 splice variant and, thus, are characterized by diminished signal transduction responses.[11] In recent studies, clinical symptoms such as unexplained abdominal symptoms and meal-unrelated dyspepsia in FD have also been associated with the various polymorphism including GNβ3 polymorphism.[12,13] Moreover, familial clustering of FD has been reported, suggesting that a genetic factor may also play a significant role in developing FD.[14]

In addition, serotonin (5-hydroxytryptamine; 5-HT) as well as G-protein is key signaling molecule sensorimotor functions in the GI tract. 5-HT_{1A} receptor agonists with anxiolytic properties delays gastric emptying[15] and relaxes the proximal stomach in human.[16] 5-HT_{2A} receptor has been reported to be involved in the modulation of enteric neuronal activity.[17] 5-HT_3 receptor agonist, MKC-733 has also delayed gastric emptying in association with relaxation of the proximal stomach. 5-HT_4 receptor as well as 5-HT_3 play an important role in GI sensory and motor functions.[18] Lelyveld *et al.* have studied whether there was a significant relationship among three genotypes including 5-HT_3 and clinical symptoms in FD patients based on Rome II classification in Austria.[19]

In this study, we aimed to clarify whether GNβ3 genotype as well as genotypes of 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} could be associated with clinical symptoms and gastric emptying via impairment of receptor dysfunction, reduction of serotonin levels, and the response for serotonin in FD patients based on Rome III classification.

MATERIAL AND METHODS

Patients

Seventy-four consecutive patients presenting with typical symptoms of FD (EPS, $n = 24$; PDS, $n = 51$) and sixty-four healthy volunteers were enrolled after upper GI endoscopy and abdominal ultrasonography. Patients were diagnosed according to Rome III criteria.[20] Healthy volunteers were also recruited from the volunteers among Japanese medical staffs of Nippon Medical School, who have no clinical history of gastroduodenal disease including clinical symptom of FD symptoms. Exclusion criteria included severe heart disease, renal or pulmonary failure, liver cirrhosis, severe systemic illness, and history of malignant disease. Patients with previous gastroduodenal surgery, duodenal ulcer scar, diabetes mellitus, and recent use of NSAIDs, PPIs or anticoagulants at endoscopy were also excluded. *Helicobacter pylori* infection was determined by both the ^{13}C-urea breath test and by histological identification. Written informed consent was obtained from all subjects prior to undergoing upper GI endoscopy and abdominal ultra-sonography for evaluation of their dyspeptic symptoms. The study protocol was approved by the Ethics Review Committee of Nippon Medical School Hospital.

Clinical symptoms

Clinical symptoms of FD were evaluated according to Rome III criteria[20] and must have included at least one of the following: bothersome postprandial fullness, early satiation, epigastric pain, or epigastric burning. Diagnostic criteria for PDS included bothersome postprandial fullness occurring after ordinary-sized meals and/or early satiation that prevented finishing a regular meal, with either symptom occurring at least several times a week. Diagnostic criteria for EPS included all of the following: pain or burning that is intermittent, localized to the epigastrium, and of at least moderate severity at least once per week. Diagnostic criteria for PDS and EPS were fulfilled with symptoms occurring for the last 3 months and the onset of symptoms occurring at least 6 months prior to diagnosis. Abdominal symptoms including the feeling of hunger were assessed by using the modified questionnaire that has been applied in previous studies.[1,21,22] We assessed abdominal symptoms including the feeling of hunger using the modified Glasgow dyspepsia severity score,[23] which consist of frequency (never; score 0, on only 1 or 2 days; score 1, on approximately 1 day per week; score 3, on approximately 50% of days; score; score 4, on most days; score 5), duration of symptoms (maximal score 5) and intensity of symptoms (maximal score 3). Status of depression was evaluated by SRQ-D (Self-Rating Questionnaire For Depression) score.[24]

Measurement of gastric motility

Sodium acetate (water soluble) for emptying of liquids was used as tracer (Cambridge Isotope Laboratories; MA, USA). Probes were analyzed by non-dispersive infrared spectroscopy (IRIS, Wagner Analyzentechnik; Bremen, Germany). The subject's own production of 300 mmol CO_2 per m^2 body surface and per hour were set as default. We used an Integrated Software Solutions program to calculate the half gastric emptying time ($T_{1/2}$) and the lag phase (T_{max}; min) as the point of maximum gastric emptying according to Hellmig *et al.*[25] T_{max} value greater than 60 min, representing the mean T_{max} in healthy volunteers plus SD, was defined to represent relative disturbances in gastric emptying according to

the diagnostic criteria of the Japan Society of Smooth Muscle Research and our study.[3,26]

Study protocol for gastric emptying of liquids

The liquid test meal consisted of 100 mg of ^{13}C-acetate dissolved in 200 mL of liquid meal (Racol, 1 mL kcal^{-1}; Otsuka Pharmacia Company, Tokyo, Japan). Breath samples were collected 0, 10 s, 5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 50 min, 60 min, 75 min, and 90 min after ingestion of the test meal at 10:00 a.m. Patients were instructed not to drink, eat, or smoke during the test.

Genotyping

We have developed or optimized the following assays for genetic variation. Genotypes were confirmed or selectively assessed for GNβ3, 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_{3A}$, 5-HT$_{4A}$ genotypes by direct sequencing using an ABI 7500 Fast. Gene polymorphisms were determined by methods in the literature. Real-time polymerase chain reaction using TaqMan chemistries (Applied Biosystems, Foster City, CA, USA) was used to determine alleles present in each sample. Real-time polymerase chain reactions were performed in an Applied Biosystems 7500 Fast machine (Applied Biosystems). TaqMan primer-probe assays for GNβ3 SNPs C825T (rs:5443; C-2184734-10), 5-HT$_{1A}$ SNPs C1019G (rs:6296; C-11904666-10), 5-HT$_{2A}$ SNPs G1438A (rs:6311; C-7488465-10), and 5-HT$_{3A}$ SNPs C42T (rs:1062613; C-2184734-10), 5-HT$_{4A}$ SNPs G353 + 6A (rs:2278392; C-15965377-10) were purchased from Applied Biosystems. In briefly, each reaction volume was 10 μl and consisted of 5 μl of a TaqMan Genotyping Master Mix (Applied Biosystems), 0.25 μl of a 40 × primer probe assay mix (Applied Biosystems), H_2O 3.75 μl and 1 μl (10 ng) genomic DNA. Amplification conditions consisted of 95 °C, 10 min; 40 cycles of: 95 °C, 15 s; 60 °C, 60 s; followed by 50 °C, 2 min. And then analyzed using automated software (SDS 2.1; Applied Biosystems) to determine the genotype of each sample.

Measurement of plasma ghrelin levels in FD patients

We measured plasma ghrelin levels to evaluate their association with polymorphism of GNβ3 825CT. Blood samples were obtained after an overnight fast of > 12 h, immediately transferred to chilled polypropylene tubes containing Na_2EDTA and aprotinin, then centrifuged at 4 °C. One tenth of the volume of 1N HCl was immediately added to the separated plasma. The acylated and des-acylated forms of ghrelin were measured using commercially available ELISA kits according to the manufacturer's instructions (Active Ghrelin ELISA Kit and Desacly-Ghrelin ELISA Kit, Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). The intra- and inter-assay coefficients of variation (CV) were 6.5% and 9.8% for acylated ghrelin, and 3.7% and 8.1% for des-acylated ghrelin.

Statistical analysis

For statistical evaluation of group data, Students' *t*-test for paired data and analysis of variance (ANOVA) for multiple comparisons were followed by Scheffe's F test. Mann–Whitney *U* test was used for analysis of categorical data. To determine factors that associated with the disturbance of gastric emptying, multiple logistic regression analysis was used at 95% confidence intervals and associated *P* values. A *P* value < 0.05 was statistically significant.

RESULTS

Characteristics of FD patients and healthy volunteers

The age, sex, and BMI in FD and healthy volunteers were not statistically different (Table 1). SRQ-D score in FD patients was also significantly higher ($P < 0.001$) compared with that of healthy volunteers. Both of $T_{1/2}$ and T_{max} values in FD patients were significantly ($P < 0.001$, $P < 0.001$) higher compared with those of healthy volunteers. The proportion of disturbed gastric emptying in FD patients (43.2%) was significantly ($P < 0.01$) higher compared with that of healthy volunteers (4.7%) (Table 1).

GNβ3, 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{3A}$, and 5-HT$_{4A}$ genotypes in FD patients

GNβ3, 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{3A}$, and 5-HT$_{4A}$ genotypes distribution in FD were 14CC (18.9%), 44CT (59.5%), 16TT (21.6%); 44GG (59.5%), 28GC (37.8%), 2CC (2.7%); 17CC (23.0%), 35CT (47.3%), 22TT (29.7%); 58CC (78.4%), 16CT (21.6%); 7AA (9.4%), 29GA (39.2%), 38GG (51.4%), respectively. Meanwhile, in the healthy controls, GNβ3, 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_{3A}$, and 5-HT$_{4A}$ genotypes distribution were 17CC (26.6%), 28CT (43.7%), 19TT (29.7%); 33GG (51.6%), 28GC (43.7%), 3CC (4.7%); 18CC (28.1%), 30CT (46.9%), 16TT (25%); 49CC (76.5%), 14CT (21.9%), 1TT (1.6%); 7AA (10.9%), 22GA (34.4%), 35GG (54.7%), respectively. Each genotype distribution was not significantly different in FD patients and healthy volunteers (Table 2).

Multiple logistic analysis for T_{max} value in FD patients

As various clinical symptoms in FD patients are partly involved in the disturbance of gastric motility, we tried

Table 1 Characteristics of the patients

	FD	Healthy volunteer
Subjects (*n*)	74	64
Age	59.2 ± 14.2	37.2 ± 9.13
Sex (M/F)	36/38	57/7
BMI	22.2 ± 2.57	22.9 ± 2.63
SRQ-D	9.94 ± 0.71	6.14 ± 0.49
$T_{1/2}$	94.5 ± 3.54	72.8 ± 1.62
T_{max}	59.2 ± 1.74	46.7 ± 0.95
Disturbed gastric emptying (%)	43.2	4.7

FD, functional dyspepsia.

Table 2 GNβ3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A} and 5-HT_{4A} genotypes in FD patients

	Genotype				
Variables *n* (%)	CC	CT	TT	OR CC *vs* others	*P* value
GNβ3-G825C polymorphism and FD					
Healthy volunteers (*n* = 64)	17 (26.6)	28 (43.7)	19 (29.7)	Reference	
FD (*n* = 74)	14 (18.9)	44 (59.5)	16 (21.6)	0.645	0.283
	Genotype				
Variables *n* (%)	GG	GC	CC	OR GG *vs* others	*P* value
5-HT_{1A}-C1019G polymorphism and FD					
Healthy volunteers (*n* = 64)	33 (51.6)	28 (43.7)	3 (4.7)	Reference	
FD (*n* = 74)	44 (59.5)	28 (37.8)	2 (2.7)	1.38	0.316
	Genotype				
Variables *n* (%)	CC	CT	TT	OR CC *vs* others	*P* value
5-HT_{2A}-G1438A polymorphism and FD					
Healthy volunteers (*n* = 64)	18 (28.1)	30 (46.9)	16 (25.0)	Reference	
FD (*n* = 74)	17 (23.0)	35 (47.3)	22 (29.7)	0.762	0.488
	Genotype				
Variables *n* (%)	CC	CT	TT	OR CC *vs* others	*P* value
5-HT_{3A}-G42T polymorphism and FD					
Healthy volunteers (*n* = 64)	49 (76.5)	14 (21.9)	1 (1.6)	Reference	
FD (*n* = 74)	58 (78.4)	16 (21.6)	0 (0)	1.11	0.798
	Genotype				
Variables *n* (%)	AA	GA	GG	OR AA *vs* others	*P* value
5-HT_{4A}-G353 + 6A polymorphism and FD					
Healthy volunteers (*n* = 64)	7 (10.9)	22 (34.4)	35 (54.7)	Reference	
FD (*n* = 74)	7 (9.4)	29 (39.2)	38 (51.4)	1.35	0.825

to clarify whether these parameters including age, BMI, sex, SRQ-D score, FD symptoms, *H. pylori* infection, and five genotypes are linked to T_{max} value as a marker of gastric emptying. Multiple logistic regression analysis revealed that there was no significant relationship between these parameters and T_{max} value in FD patients (Table 3).

GNβ3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} genotypes in PDS patients with or without impaired gastric emptying

We then compared five genotypes (GNβ3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A}) in FD patients with or without disturbance of gastric emptying. We divided FD patients into two groups which are disturbed with gastric emptying (T_{max} value > 60 min) and normal gastric emptying (T_{max} value < 60 min). We could not find a significant correlation ($P = 0.620$; $P = 0.760$; $P = 0.365$; $P = 0.570$; $P = 0.691$) between T_{max} value and genotype of GNβ3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} in seventy-four FD patients, respectively. The proportion of GNβ3 825C/C in FD patients with disturbed gastric emptying was relatively greater ($P = 0.06$) compared with that of healthy volunteers (data not shown).

To investigate whether there is any significant difference in five genotypes and T_{max} value as a marker of gastric emptying in PDS patients, we compared genotypes with PDS patients with or without disturbed gastric emptying. We found that there was no significant relationship ($P = 0.501$; $P = 0.131$; $P = 0.924$; $P = 0.490$; $P = 0.390$) between five genotypes and PDS patients with impaired gastric emptying, respectively (Table 4).

Table 3 Multiple logistic analysis for impaired T_{max} value in FD patients (n = 74)

Factor	Odds ratio (95% CI)	P value
Age	1.034 (0.998–1.071)	0.061
BMI	1.032 (0.857–1.242)	0.742
SEX	1.100 (0.438–2.761)	0.839
SRQ-D	0.993 (0.919–1.074)	0.897
Heartburn	0.955 (0.899–1.016)	0.146
PDS-like*	1.957 (0.639–5.992)	0.239
EPS-like†	0.796 (0.208–3.046)	0.739
H. pylori	1.821 (0.273–3.787)	0.271
GNβ3 (CC; CT/TT)	1.400 (0.436–4.496)	0.577
5-HT_{1A} (GG; GC/CC)	0.791 (0.310–2.017)	0.624
5-HT_{2A} (CC; CT/TT)	1.957 (0.639–5.992)	0.239
5-HT_{3A} (CC; CT/TT)	1.916 (0.591–6.214)	0.280
5-HT_{4A} (AA; GA/GG)	2.027 (0.206–4.928)	0.418

FD, functional dyspepsia; PDS, postprandial distress syndrome; EPS, epigastric pain syndrome.
*Most bothersome symptom based on physician interview was early satiety.
†Most bothersome symptom based on physician interview was upper abdominal pain.

Table 4 Association between GNβ3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} polymorphism and gastric emptying in PDS patients

Genotypes	T_{max} > 60 min	T_{max} < 60 min	OR (95% CI)	P value
GNβ3 CC	6	5	2.00 (0.519–7.7703)	0.501
CT/TT	15	25		
5-HT_{1A} GG	17	17	3.25 (0.879–12.00)	0.131
GC/CC	4	13		
5-HT_{2A} CC	6	7	1.31 (0.368–4.663)	0.924
TT/CT	15	23		
5-HT_{3A} CC	18	22	2.18 (0.503–9.442)	0.490
CT/TT	3	8		
5-HT_{4A} AA	2	4	0.37 (0.061–2.232)	0.390
GA/GG	26	19		

PDS, postprandial distress syndrome.

Table 5 Association between GNβ3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} polymorphism and gastric emptying in PDS patients without reflux

Genotypes	T_{max} > 60 min	T_{max} < 60 min	OR (95% CI)	P value
GNβ3 CC	6	3	5.71 (1.117–2.918)	0.045
CT/TT	7	20		
5-HT_{1A} GG	8	13	1.6 (0.399–6.414)	0.953
GC/CC	5	10		
5-HT_{2A} CC	5	6	0.63 (0.147–2.697)	0.690
TT/CT	8	17		
5-HT_{3A} CC	12	16	5.25 (0.567–48.58)	0.213
CT/TT	1	7		
5-HT_{4A} AA	1	3	0.43 (0.040–4.593)	0.625
GA/GG	14	18		

PDS, postprandial distress syndrome.

GNβ3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} genotypes in PDS patients without gastro-esophageal reflux symptom

Moreover, to investigate whether there is any significant relationship between five genotypes and T_{max} value in PDS patients without gastro-esophageal reflux symptom, we compared five genotypes with PDS patients without gastro-esophageal reflux symptom with or without impaired gastric emptying in similar way. We confirmed that there was a significant relationship (P = 0.045) between GNβ3 825CC genotype and PDS patients without gastro-esophageal reflux symptom accompanying impaired gastric emptying (Table 5). In contrast, there were no significant relationship between 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} genotypes and PDS patients without gastro-esophageal reflux symptom accompanying with impaired gastric emptying (P = 0.953; P = 0.690; P = 0.213; P = 0.625) (Table 5).

Association between GNβ3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} genotypes and clinical symptoms in FD patients

As GNβ3 genotype has been previously reported to be linked to clinical symptoms in FD patients, we also determined whether five genotypes including GNβ3 are associated with several clinical symptoms in Japanese FD patients based on Rome III classification. In our FD populations, GNβ3 825CC genotype in FD patients is significantly (P = 0.0485) associated with the feeling of hunger compared with GNβ3 825CT and TT genotypes (Fig. 1).

As ghrelin levels have been reported to be associated with appetite, we tried to determine whether plasma ghrelin levels are linked to GNβ3 825CC genotype in FD patients. We measured both plasma acylated ghrelin (7.08 ± 0.63 fmol mL^{-1}) and des-acylated ghrelin levels (74.7 ± 6.22 fmol mL^{-1}) in FD patients. There was no significant difference (P = 0.269) in acylated ghrelin levels in GNβ3 825CC and GNβ3 825CT/TT genotypes.

In contrast, there are not significant differences between 5-HT_{1A} GG, 5-HT_{2A} CC, 5-HT_{3A} CC, and 5-HT_{4A} AA genotypes and clinical symptoms compared with other genotypes, respectively (Fig. 1).

DISCUSSION

The major findings of this study are (i) There was a significant relationship between GNβ3 825CC genotype and PDS patients without gastro-esophageal reflux

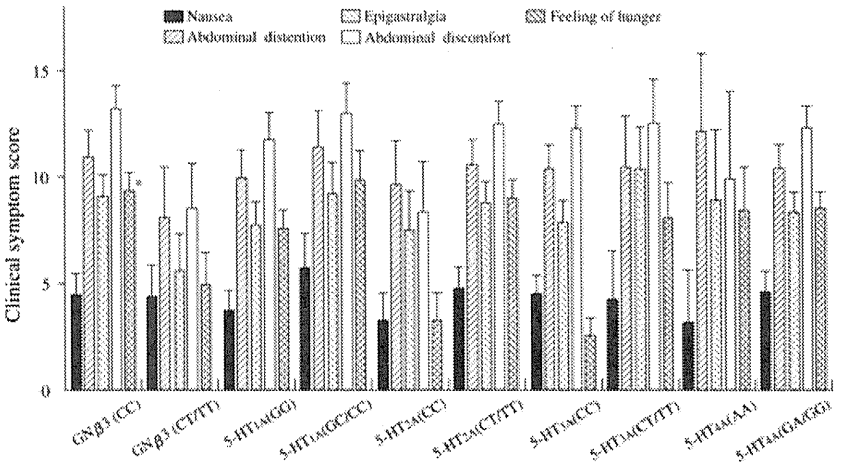

Figure 1 Association between GNβ3, 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} genotypes and gastrointestinal symptoms in FD patients. There was no significant relationship between 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, 5-HT_{4A}, and various clinical symptoms. In Japanese, GNβ3 825CC genotype in FD patients is significantly (P = 0.0485) associated with the feeling of hunger compared with GNβ3 825CT and TT genotypes. GNβ3: 14CC, 60CT/TT; 5-HT_{1A}: 44GG, 30GC/CC; 5-HT_{2A}: 17CC, 57CT/TT; 5-HT_{3A}: 58CC, 16CT/TT; 5-HT_{4A}: 7AA, 67GA/GG. * *vs* GNβ3 825CT/TT genotypes. FD, functional dyspepsia.

symptoms with impaired gastric emptying, (ii) GNβ3 825CC genotype in FD patients was significantly associated with the feeling of hunger symptom compared with GNβ3 825CT and TT genotypes.

There is increasing evidence that susceptibility to functional GI disorders is also influenced by hereditary factors.[27–29] In this study, we have first reported that GNβ3 CC variant was significantly associated with disturbance of gastric emptying in PDS patients without gastro-esophageal reflux symptoms. Previous studies have reported that there is reasonable evidence that the GNβ3 status is associated with depression,[30] increased immune cell activation,[11] and altered activation of α2-adrenoreceptors.[31] In addition, Holtmann *et al.* have reported that the homozygous GNβ3 825CC was associated with upper abdominal symptoms unrelated to meals in Germany,[12] while previous studies have shown that the homozygous GNβ3 825CC or 825TT was also associated with meal-unrelated dyspepsia in people randomly selected from the US community and EPS patients in Japan.[13,32] Recent study has reported that there was no significant relationship between gastric emptying and GNβ3 genotype in Rome II-based FD patients.[33] In this study, we investigated the relationship between gastric emptying and the GNβ3 subunit 825 genotype among FD, PDS, and healthy volunteers based on Rome III classification. We have previously reported that the T_{max} value as a marker of gastric emptying in PDS patients was significantly greater compared with that of healthy volunteers.[3] Therefore, in our study, we focused on the GNβ3 genotype in PDS patients with or without impaired gastric emptying and found a significant relationship between GNβ3 825CC genotype and impaired gastric emptying in PDS patients without gastro-esophageal reflux symptoms. In addition, because of small number of subjects of Rome III subgroups, type II error could not be excluded. Thus, our result should be treated carefully with caution until replicated.

The age of onset of gastro-esophageal reflux disease (GERD) is variable and many individuals develop the disease during childhood. Gastro-esophageal reflux disease is the most common esophageal disorder of children, affecting about 11% of all infants during their first year of life.[34] Epidemiological data justify theory formation about a genetic component in the pathophysiology of GERD. The disease etiology is further complicated by a substantial genetic contribution as shown by familial clustering,[35] autosomal dominant familial transmission of disease[36,37] as well as twin studies.[38] As Vries *et al.* have reported that GERD is associated with the GNβ3 825CT genotype,[39] we investigated whether the GNβ3 825CC variant were associated with disturbance of gastric emptying in PDS patients without gastro-esophageal reflux symptoms in this study. Further studies are needed to clarify whether the reduction of threshold of 5-HTs receptors is associated with reflux symptom through protein kinase-mediated signaling pathways induced by impairment of G-protein-coupled receptors (GPCRs) via GNβ3 825CT variant in these patients.

Considering that there were the discrepancy about clinical symptoms and gastric motility for GNβ3 825 alleles of FD patients in several countries,[12,13,19,32,33] it seems to be a very important factor that Japanese patients with *H. pylori*-infected gastritis have low levels of acid secretion compared with Europeans and Americans.[40] It is very critical issue about the relationship between gastric acidity and gastric motility because Lee *et al.* and Schwartz *et al.* have reported that intraduodenally administrated acid affects gastroduodenal motility as well as visceral hypersensitivity.[41,42] Considering these previous studies, high

prevalence of *H. pylori* infection may be considered to play an important role in the etiology of certain FD patients in Japan.[43] Saito *et al.* have reported that acceleration of gastric emptying was observed in *H. pylori*-infected animal model.[44] In our study, the proportion of *H. pylori*-infected PDS patients was 37%. In our previous study, *H. pylori* infection reduced ghrelin-producing cell numbers which are linked to gastric emptying.[45] Therefore, in the future study, we should investigate whether GNβ3 825 genotype may be linked to gastric emptying among *H. pylori*-negative subjects. Our findings thus needed to be replicated in different populations and other races. On the other hand, the allele distribution in controls was very similar to allele distributions that have been observed in previous studies of Japanese.[32,46] Oshima *et al.* and Tahara *et al.* have reported GNβ3 825TT genotype is associated with EPS-like dyspepsia or dyspepsia, respectively.[32,46] This discrepancy between these reports and our results may have occurred in sample selection, such as patient's age, psychological condition (SRQ-D score is high), Rome III-categorized patients, and visiting care centers. The novel 5-HT_{1A} agonist R137696 has been reported to affect the proximal gastric function[16] as well as previous studies.[47,48] 5-HT_{3A} receptors also seem to be involved both in the transmission of the sensation that arises from the stomach and in the process of gastric emptying and accommodation.[49] However, we have first compared genotypes of 5-HTs with gastric emptying in Rome III-based FD patients. We could not find any significant relationship between genotypes of 5-HTs such as 5-HT_{1A}, 5-HT_{2A}, 5-HT_{3A}, and 5-HT_{4A} and gastric emptying in these FD patients.

In the present data, there was a significant relationship between the feeling of hunger and GNβ3 825CC genotype in Rome III-based FD patients. In contrast, in Japanese patients, Tahara *et al.* have reported that the homozygous 825T allele of the GNβ3 protein influences the susceptibility of Japanese to dyspepsia.[47] In our data, we could not find any significant relationship between the GNβ3 825CC genotype and Rome III-based symptoms, such as abdominal distention, epigastralgia, and abdominal discomfort. However, we investigated that there was a significant relationship between the feeling of hunger and the GNβ3 825CC genotype in FD patients. We could not find a significant relationship between disturbed T_{max} value and the feeling of hunger ($P = 0.608$) or early satiety ($P = 0.239$) in FD patients using multiple logistic analysis. In contrast, Stanghellini *et al.* have reported that disturbed gastric emptying is associated with satiation and impaired food intake.[7] We have previously reported that there was a significant relationship between low level of acylated ghrelin linked to appetite and T_{max} value.[3] However, in our data, the score for feeling of hunger was not significantly ($P = 0.473$) associated with acylated-ghrelin levels. In addition, there was also no significant difference ($P = 0.269$) in acylated ghrelin levels in GNβ3 825CC and GNβ3 825CT/TT genotypes. Further studies are needed to clarify the mechanism by which the GNβ3 825CC genotype is associated with the feeling of hunger in *H. pylori*-negative FD patients.

Taken together, in this study, we determined that there was a significant relationship between impairment of gastric emptying and the GNβ3 825CC genotype in Rome III-based PDS patients without gastro-esophageal reflux symptoms. Further studies are needed to clarify whether the GNβ3 825CC genotype are linked to disturbance of gastric emptying and feeling of hunger via diminished transduction responses.

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The effects of nizatidine on transient lower esophageal sphincter relaxations (TLESRs) and acid reflux in healthy subjects

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Received August 24, 2011; Accepted September 30, 2011

Abstract

Background: A study in Japan has found that nizatidine (NIZ) is more effective than other histamine H2 receptor agonists (H2RAs) in treating reflux esophagitis (RE), although the NIZ group included a greater number of patients with severe RE. As there was no difference in the level of acid suppression among H2RAs, it is possible that NIZ has other effects on esophageal acid exposure (EAE) besides acid suppression. In this study, the effect of NIZ on transient lower esophageal sphincter relaxations (TLESRs) and acid reflux was evaluated in healthy subjects. Methods: In 10 healthy subjects, while in a sitting position, esophageal motility and a pH study were measured for 3 hours after a meal on 2 separate days at least 2 weeks apart. Participants received an oral dose of 150 mg of NIZ, 60 min before the meal on one day and a placebo on the other. Both studies were preceded by a week of treatment with either NIZ (150 mg, bid) or a placebo and the order of treatment was randomized. Results: Basal LES pressure in the NIZ group (14.1 mmHg, median) was significantly greater than that of the placebo group (8.5 mmHg). The rate of TLESRs in the NIZ group (22.0/3 h) for the postprandial 3-hour period was significantly less than that of the placebo group (16.5/3 h) and the rate of acid reflux during TLESRs (24.7%) and the EAE (0.2%) in the NIZ group for the postprandial 3-hour period was also significantly less than that of the placebo group (74.4% and 2.8%, respectively). Conclusion: NIZ significantly reduces acid reflux by inhibiting both the rate of TLESRs and acid reflux during TLESRs.

Key words: nizatidine, transient lower esophageal sphincter relaxation, esophageal acid exposure, acid reflux

Introduction

A study carried out in Japan has found that nizatidine (NIZ) 150 mg bid, is more effective than other histamine H2 receptor agonists (H2RAs) in treating reflux esophagitis (RE), although the

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