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Original Article

Leu72Met408 Polymorphism of the Ghrelin Gene Is Associated With Early Phase of Gastric Emptying in the Patients With Functional Dyspepsia in Japan

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Background/Aims
There are no available data about the relationship between ghrelin gene genotypes and early phase of gastric emptying in functional dyspepsia (FD) as defined by Rome III classification.

Methods
We enrolled 74 patients presenting with typical symptoms of FD and 64 healthy volunteers. Gastric motility was evaluated using the ^{13}C-acetate breath test. We used Rome III criteria to evaluate upper abdominal symptoms and self-rating questionnaires for depression (SRQ-D) scores to determine status of depression. The Arg51Gln (346G>A), preproghrelin (3056T>C), Leu72Met (408C>A), Gln90Leu (3412T>A) and G-protein β3 (825C>T) polymorphisms were analyzed in the DNA from blood samples of enrolled subjects. Genotyping was performed by polymerase chain reaction.

Results
There was a significant relationship between the Gln90Leu3412 genotype and SRQ-D score in FD patients (P = 0.009). Area under the curve at 15 minutes (AUC_{15}) value was significantly associated with the Leu72Met408 genotype (P = 0.015) but not with entire gastric emptying.

Conclusions
The Leu72Met (408C>A) single nucleotide polymorphism was significantly associated with early phase of gastric emptying in FD patients. Further studies will be necessary to clarify the association between ghrelin gene single nucleotide polymorphisms and early phase of gastric emptying in FD patients.
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Key Words
Depression; Gastric emptying; Ghrelin; Polymorphism, genetic

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Introduction

Ghrelin (growth hormone secretagogue receptor ligand, GHRL) has been recognized as an endogenous ligand for the growth hormone secretagogue receptor in the oxyntic gland of the stomach.[1] The plasma GHRL concentration rises during fasting and falls quickly after meals. In rodents, central or peripheral administration of GHRL stimulated gastric contraction and emptying[2] and showed prokinetic effects in a postoperative ileus model in rats.[3] Previous studies have reported that delayed gastric emptying was observed in 30% of the patients with functional dyspepsia (FD).[4,5] In addition, Kusano et al[6] have reported that rapid gastric emptying might be a more important factor than delayed gastric emptying in patients with FD. Therefore, it may be very important for consideration of the pathophysiology of FD patients to determine the precise mechanisms of disturbances of gastric emptying in early postprandial phase. In pharmacologic studies, acylated ghrelin has been shown to accelerate gastric emptying, increase gastric tone, and induce premature inter-digestive migrating motor complex activity. In addition, an increase in ghrelin occurs not only in response to states of energy insufficiency[7] but also against stress.[8] Therefore, human subjects suffering from acute psychosocial stress also display increased plasma ghrelin.[9] Asakawa et al[10] have reported that both central and peripheral administration of ghrelin is a potent inducer of anxiogenic behavior in mice. Carlini et al[11] have also reported that ghrelin induces anxiogenesis in rats.

There are increasing evidences that susceptibility to functional gastrointestinal disorders (FGIDs) is also influenced by hereditary factors.[12,13] Familial clustering of FD has been recently reported and several genotypes associated with FD have been reported.[14-17] Holtmann et al[15] have reported that the homozygous G-protein β3 subunit 825CC (GNβ3825CC) genotype was associated with upper abdominal symptoms unrelated to meals in Germany. However, recent studies on the association between GNβ3 polymorphism and FGIDs are very heterogenous and contradictory.[16,17] The GHRL gene codes for 2 short hormones, ghrelin and obestatin, which are associated with gastric motility. Although ghrelin is a potent stimulator of food intake and gastrointestinal motility, the effects of obestatin on food intake and gastrointestinal motility have been controversial.[18] The gene encoding preproghrelin, the GHRL precursor, is located on chromosome 3 (3p25-26) and is comprised of 4 exons and 3 introns.[19] Four nonsynonymous single nucleotide poly-morphisms (SNPs) of the GHRL gene (Arg51Gln, Leu72Met, preproghrelin3056, and Gln90Leu) have been reported. The Arg51Gln SNP has been associated with lower GHRL and insulin-like growth factor I concentrations.[19] The Leu72Met SNP has been linked to obesity-related phenotypes, but the findings are controversial.[19-21] In a previous study, we found a correlation between the preproghrelin 3056TT genotype and low acylated ghrelin levels in *Helicobacter pylori*-negative FD patients.[22]

In this study, we aimed to investigate whether GHRL and GNβ3 gene SNPs might contribute to early phase of gastric emptying and psychogenic state in Rome III-based FD patients.

Materials and Methods

Subjects

Seventy-four patients presenting with typical symptoms of FD and 64 healthy volunteers were enrolled after upper gastrointestinal endoscopy and abdominal ultrasonography. Patients were diagnosed according to Rome III criteria.[23] Healthy volunteers were also recruited from volunteers among Japanese medical staffs of the Nippon Medical School, who had no clinical history of gastroduodenal disease including clinical symptoms of FD. Exclusion criteria included severe heart diseases, renal or pulmonary failure, liver cirrhosis, severe systemic illness and history of malignant diseases. Patients with previous gastroduodenal surgery, duodenal ulcer scar, diabetes mellitus, and recent use of non-steroidal anti-inflammatory drugs (NSAIDs), proton pump inhibitors (PPIs) or anticoagulants at endoscopy were also excluded. Written informed consent was obtained from all subjects prior to undergoing upper gastrointestinal endoscopy and abdominal ultrasonography 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.[23] Diagnostic criteria for postprandial distress syndrome (PDS) included bothersome postprandial fullness occurring after ordinary-sized meals and/or early satiation that prevents completion of a normal meal, with either symptom occurring at least several times a week. Determination of diagnosis for epigastric pain syndrome (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. We as-

sessed abdominal symptoms using the modified Glasgow dyspepsia severity score,[24] which consists of frequency of dyspeptic symptoms over the past 6 months (never, score 0; on only 1 or 2 days, score 1; on approximately 1 day per month, score 2; on approximately 1 day per week, score 3; on approximately 50% of days, score 4; and on most days, score 5), duration of symptoms (never, score 0; within 1 hour, score 1; on 1-3 hours, score 2; on 3-6 hours, score 3; on half a day, score 4; on all day, score 5) and intensity of symptoms (mild, score 1; moderate, score 2; and severe, score 3). The feeling of hunger was evaluated by frequency as follows: never, score 5; on only 1 or 2 days per 3 months, score 4; on approximately 1 day per month, score 3; on 1 day per week, score 2; on half a day, score 1; and on all day, score 0.[22,25] Status of depression was evaluated by the self-rating questionnaire for depression (SRQ-D) score.[26]

Measurement of Gastric Motility

Sodium acetate (water-soluble) for emptying of liquids was used as tracer (Cambridge Isotope Laboratories, Cambridge, MA, USA). The liquid test meal consisted of 100 mg of ^{13}C-acetate dissolved in 200 mL of a liquid meal (Racol, 1 mL/kcal; Otsuka Pharmacia Company, Tokyo, Japan). Breath samples were collected 0 and 10 seconds and 5, 10, 15, 20, 30, 40, 50, 60, 75 and 90 minutes after ingestion of the test meal at 10 AM. Patients were instructed not to drink, eat or smoke during the test. 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 was set as default. We used an integrated software solution program to calculate the half gastric emptying time ($T_{1/2}$) and the lag phase (T_{max}, minute) as the point of maximum gastric emptying according to Hellmig et al.[27] The $T_{1/2}$ was calculated as previously described.[27,28] The $T_{1/2}$ represents the time when 50% of the initial gastric content was emptied. A T_{max} value of > 65 minutes, representing the mean T_{max} in healthy volunteers plus 2 standard deviation (SD), was defined to represent disturbances in gastric emptying according to the diagnostic criteria of the Japan Society of Smooth Muscle Research.

Data Analysis

The time plot of pulmonary [$^{13}CO_2$] excretion (% dose/hr) was fitted to the function:

$$(\% \text{ dose/hr}) = m \times k \times \beta \times e^{-kt} \times (1\text{-}e^{-kt})^{\beta-1}$$

where m is the cumulative [$^{13}CO_2$] recovery at the infinite time, t is in hours and k and β are regression estimated constants.

$$(\text{Cumulative \% dose}) = m \times (1\text{-}e^{-kt})$$
$$AUC_5 = m \times (1\text{-}e^{-k \times 0.08})^{\beta} \quad [T: 5 \text{ minutes} = 0.08 \text{ hours}]$$
$$AUC_{15} = m \times (1\text{-}e^{-k \times 0.25})^{\beta} \quad [T: 15 \text{ minutes} = 0.25 \text{ hours}]$$
$$AUC_{30} = m \times (1\text{-}e^{-k \times 0.5})^{\beta} \quad [T: 30 \text{ minutes} = 0.5 \text{ hours}]$$
$$AUC_{60} = m \times (1\text{-}e^{-k \times 1.0})^{\beta} \quad [T: 60 \text{ minutes} = 1.0 \text{ hours}]$$
$$AUC_{90} = m \times (1\text{-}e^{-k \times 1.5})^{\beta} \quad [T: 90 \text{ minutes} = 1.5 \text{ hours}]$$

We determined area under the curve at 5 minutes (AUC_5) and AUC_{15} values as markers of the early phase of gastric emptying based on previous studies.[6,16,25,29,30] AUC_5 values of > 17.4 and AUC_{15} values of > 39.6, representing the mean AUC value of healthy volunteers plus 2SD, were defined to represent disturbances in early phase of gastric emptying.

Genotyping

We have developed or optimized the following assays for genetic variation. Genomic DNA was isolated from whole blood using a QIAamp DNA blood minikit (Qiagen, Hilden, Germany). Genotypes were confirmed or selectively assessed for GHRL (Arg51Gln346G > A [rs34911341], preproghrelin 3056T > C [rs2075356], Leu72Met408C > A [rs696217] and Gln90Leu3412T > A [rs4684677]) genotypes and the GNβ3 genotype (GNβ3C825T [rs5443]) by direct sequencing using an ABI 7500 Fast (Applied Biosystems, Foster City, CA, USA). Real-time polymerase chain reaction using TaqMan chemistries (Applied Biosystems) was used to determine alleles present in each sample. Real-time polymerase chain reactions were performed in an Applied Biosystems 7500 Fast machine. The data was analyzed using automated software (SDS 2.1; Applied Biosystems) to determine the genotype of each sample.

Statistical Methods

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. The distribution of alleles at each locus was assessed using the χ^2 statistic of the Hardy-Weinberg equilibrium. To determine factors associated with the disturbance of gastric emptying, multiple logistic regression analysis was used at 95% confidence intervals and associated P-values. Data analyses were performed by using a standard software package (SPSS version 13.0, SPSS Inc, Chicago, IL, USA). A P-value of less than 0.05 was considered statistically significant.

Power of the Study

In this study, we aimed to assess the potential association between AUC_{15} value and symptoms of FD in patients with the Leu72Met408 SNP of the GHRL gene. In our study, we determined the sample size using the PS (power and sample size calculations program) software program, a gift from Vanderbilt University. The deviation of the AUC_{15} value in healthy volunteers was approximately 3.8 ($\sigma = 3.8$). Using the above data and settings of $\alpha = 0.05$, $\beta = 0.80$, effect size $= 0.641$ and an estimated mean AUC_{15} value of 42.507 (% dose) in patients with FD, 64 healthy volunteers and 74 FD patients were determined to be sufficient to identify clinically relevant difference.

Results

Characteristics of Functional Dyspepsia Patients and Healthy Volunteers

The age, sex and body mass index (BMI) (28-74 years, M/F: 39/35, 22.18 $\pm$ 0.29 kg/m^2) of FD patients did not differ statistically from those of healthy volunteers (22-68 years, M/F: 34/30, 22.9 $\pm$ 0.34 kg/m^2). However, the SRQ-D scores, $T_{1/2}$ and T_{max} values in FD patients were significantly higher compared to those in healthy volunteers (9.94 $\pm$ 0.71 vs 6.14 $\pm$ 0.49, 94.9 $\pm$ 3.55 vs 72.8 $\pm$ 1.62, and 59.2 $\pm$ 1.74 vs 46.7 $\pm$ 0.95, respectively; all $P < 0.001$). The mean score of clinical symptoms (Glasgow) in FD patients was significantly higher compared to that in healthy volunteers (8.38 $\pm$ 0.48 vs 1.92 $\pm$ 0.30, $P < 0.001$).

G-protein β3 and Ghrelin Gene Genotypes in Functional Dyspepsia Patients

The Arg51Gln (346G>A), Leu72Met (408C>A), preproghrelin (3056T>C), Gln90Leu (3412T>A) and GNβ3 (825C>T) genotype distribution in FD patients was 72 CC (97.3%), 2 CT (2.7%); 21 GT (28.4%), 52 GG (70.3%), 1 TT (1.3%); 3 CC (4.1%), 29 TC (39.2%), 42 TT (56.7%); 71 TT (95.9%), 3 TA (4.1%); and 14 CC (19%), 46 CT (62%), 14 TT (19%), respectively. Meanwhile, in healthy controls, the Arg51Gln (346G>A), Leu72Met (408C>A), preproghrelin (3056T>C), Gln90Leu (3412T>A) and GNβ3 (825C>T) genotype distribution was 64 CC (100%); 22 GT (34.4%), 39 GG (60.9%), 3 TT (4.7%); 5 CC (7.8%), 30 TC (46.9%), 29 TT (45.3%); 58 TT (93.7%), 6 TA (6.3%); and 16 CC (25%), 29 CT (45%), 19 TT (30%), respectively. There was no significant difference between the genotype distributions of FD patients and those of healthy volunteers.

Table 1. Association Between G-protein β3 Single Nucleotide Polymorphism, and the Ghrelin Gene Single Nucleotide Polymorphisms and Self-rating Questionnaire for Depression Score in Functional Dyspepsia Patients and Healthy Volunteers

Genotype	SRQ-D score			
	FD	HV	P-value[a]	P-value[b]
Arg51Gln (346G>A)				
CC	9.87	6.14 $\pm$ 0.49	0.205	
CT/TT	9.50	0.00 $\pm$ 0.00		
Preproghrelin (3056T>C)				
TT	10.73	6.31 $\pm$ 0.60	0.178	0.357
TC/CC	8.97	6.00 $\pm$ 0.60		
Leu72Met (408C>A)				
GG	5.85	6.44 $\pm$ 0.69	0.814	0.901
GT/TT	6.53	5.68 $\pm$ 0.62		
Gln90Leu (3412T>A)				
TT	5.83	6.15 $\pm$ 0.511	$P = 0.0097$	0.921
TA/AA	21.0	6.00 $\pm$ 1.78		
GNβ3 (825C>T)				
CC	10.29	7.06 $\pm$ 1.33	0.638	0.416
TC/TT	6.17	5.81 $\pm$ 0.46		

[a]P-value refer to the difference in self-rating questionnaire for depression (SRQ-D) score of each genotype in functional dyspepsia (FD) patients.
[b]P-value refer to the difference in SRQ-D score of each genotype in healthy volunteers (HV).

Relationship Between Genotypes of Ghrelin and G-protein β3 Gene Single Nucleotide Polymorphisms and Self-rating Questionnaires for Depression Scores in Functional Dyspepsia Patients and Healthy Volunteers

To investigate whether genotypes of the GHRL and GNβ3 genes are associated with the SRQ-D score, we compared the relationship between the four GHRL gene SNPs and the GNβ3 SNP with SRQ-D scores in FD patients and healthy volunteers. SRQ-D scores in the Arg51Gln346CC genotype (dominant model) in FD patients and healthy volunteers were in similar levels to those in the Arg51Gln346CT/TT genotype (Table 1). SRQ-D scores in the preproghrelin 3056TT genotype (dominant model) in FD patients were also similar to those in the preproghrelin 3056TC/CC genotype. SRQ-D scores in the Gln90Leu3412TT genotype (dominant model) in FD patients were significantly lower than those in the Gln90Leu3412TA/AA genotype (5.83 ± 0.69 vs $21.0 \pm 2.65, P = 0.0097$). There was a significant relationship ($P < 0.01$) between the Gln90Leu3412 genotype and the SRQ-D score in FD patients (Table 1). There was no significant association between any of the four GHRL SNPs or the GNβ3 gene SNPs and SRQ-D score in healthy volunteers (Table 1).
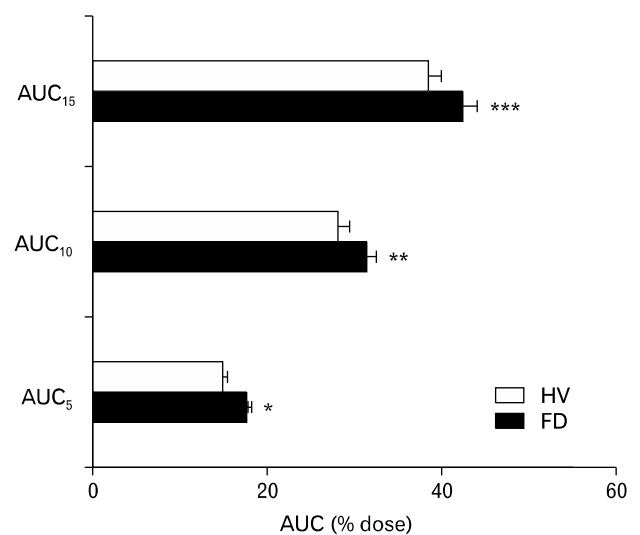
Comparison of 4 Ghrelin and G-protein β3 Gene Single Nucleotide Polymorphisms With Gastric Emptying in Functional Dyspepsia Patients and Healthy Volunteers

To investigate whether GHRL and GNβ3 gene SNPs were associated with entire gastric emptying in FD patients, we compared these SNPs with the T_{max} value as a marker of entire gastric emptying. The Arg51Gln ($346G > A$), preproghrelin ($3056T > C$), Leu72Met ($408C > A$), Gln90Leu ($3412T > A$) and GNβ3 ($825C > T$) gene SNPs were not significantly associated with the T_{max} value in FD patients ($P = 0.122, P = 0.203, P = 0.178, P = 0.513$, and $P = 0.449$, respectively) (Table 2). Then, to investigate whether the GHRL and GNβ3 gene SNPs correlated with early phase of gastric emptying, we compared them with AUC_5, AUC_{10} and AUC_{15} values as the marker of early phase of gastric emptying. AUC_5, AUC_{10}, and AUC_{15} values in FD patients were significantly greater compared to those in healthy volunteers ($P = 0.001, P = 0.015$, and $P = 0.021$, respectively) (Fig. 1). There was no significant relationship between the GNβ3 825 genotype and AUC values in healthy volunteers or in FD patients (Fig. 2). In addition, there was a significant relationship between the Leu72Met408 genotype and AUC_{10} and AUC_{15} values in FD patients ($P = 0.038$ and $P =$

Table 2. Comparison of T_{max} Value With Genotypes of Ghrelin and G-protein β3 Gene in Functional Dyspepsia Patients

| Genotypes	T_{max} > 65 min	T_{max} ≤ 65 min	P-value
Arg51Gln ($346G > A$)			0.122
CC	21	52	
CT/TT	1	0	
Preproghrelin ($3056T > C$)			0.203
TT	10	32	
TC/CC	12	20	
Leu72Met ($408C > A$)			0.178
GG	13	39	
GT/TT	9	13	
Gln90Leu ($3412T > A$)			0.513
TT	22	51	
TA/AA	0	1	
GNβ3 ($825C > T$)			0.449
CC	6	10	
TC/TT	16	42	

T_{max}, the lag phase as the point of maximum gastric emptying.

Figure 1. Comparison of area under the curve (AUC) values between functional dyspepsia (FD) patients and healthy volunteers (HV). AUC_5, AUC_{10} and AUC_{15} values in FD patients were significantly greater compared to those in HV ($P = 0.001, P = 0.015$, and $P = 0.021$, respectively). *vs AUC_5 value in HV. **vs AUC_{10} value in HV. ***vs AUC_{15} value in HV.
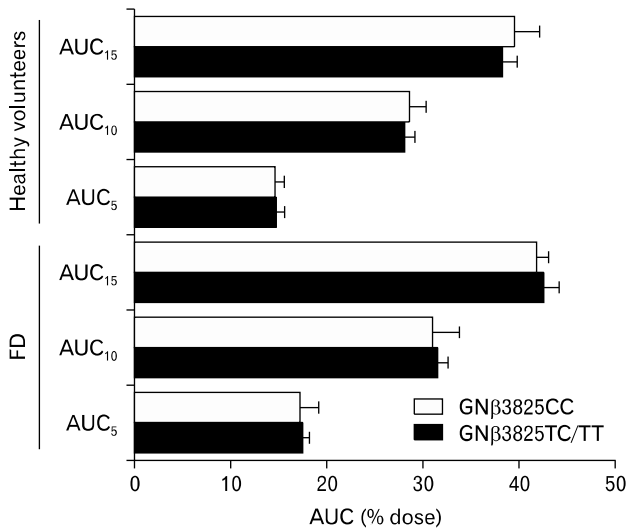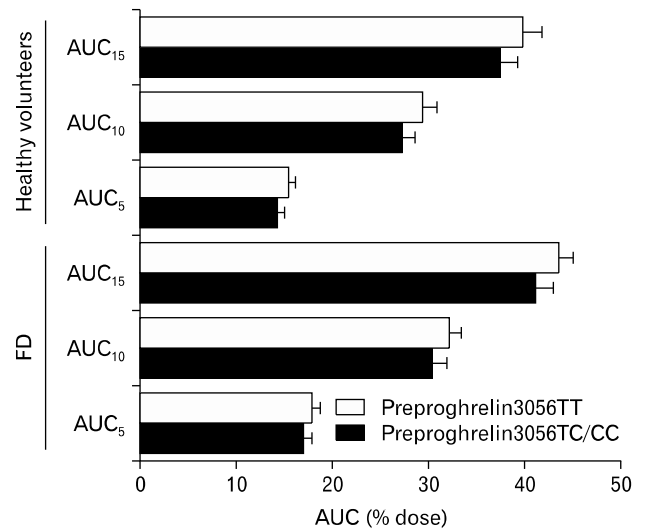
Figure 2. Comparison of area under the curve (AUC) values in the G-protein beta3 subunit (GNβ3) 825CC genotype with the GNβ3 825TC/TT genotype. AUC$_5$, AUC$_{10}$, and AUC$_{15}$ values in the GNβ3 825CC genotype (dominant model) of functional dyspepsia (FD) patients were not significantly greater compared to those in the GNβ3825TC/TT genotype.
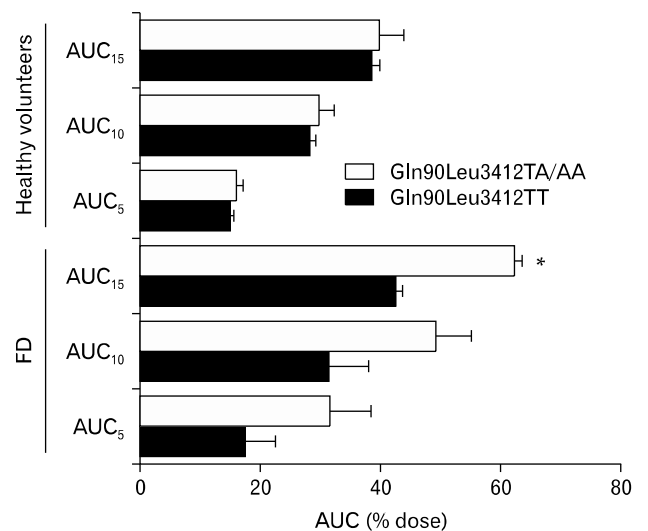

Figure 4. Comparison of area under the curve (AUC) values in the preproghrelin 3056TT genotype with the preproghrelin 3056TC/CC genotype. AUC$_5$, AUC$_{10}$, and AUC$_{15}$ values in the preproghrelin 3056TT genotype (dominant model) in functional dyspepsia (FD) patients were not significantly greater compared to those in the preproghrelin 3056TC/CC genotype.
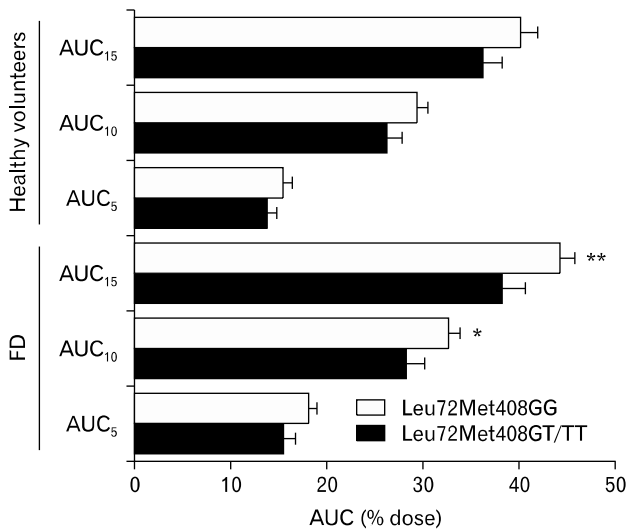
Figure 3. Comparison of area under the curve (AUC) values in the Leu72Met408GG genotype with the Leu72Met408GT/TT genotype. AUC$_{10}$ and AUC$_{15}$ values in the Leu72Met408GG (dominant model) in functional dyspepsia (FD) patients were significantly greater compared to those in the Leu72Met408GT/TT genotype. *vs. AUC$_{10}$ value in the Leu72Met408GT/TT genotype of FD patients. **vs. AUC$_{15}$ value in the Leu72Met408GT/TT genotype of FD patients.

Figure 5. Comparison of area under the curve (AUC) values in the Gln90Leu3412TA/AA genotype with the Gln90Leu3412TT genotype in functional dyspepsia (FD) patients. AUC$_{15}$ value in the Gln90-Leu3412TA/AA FD patients was significantly greater compared to that in the Gln90Leu3412TT genotype (dominant model).

0.025, respectively) (Fig. 3). However, in healthy volunteers, there was no significant relationship between the Leu72Met408 genotype and AUC values (Fig. 3). Moreover, there was no significant

relationship between the preproghrelin 3056 genotype and AUC values in either healthy volunteers or FD patients (Fig. 4). The Gln90Leu3412 genotype in FD patients was significantly associated with AUC$_{15}$ value ($P = 0.049$) (Fig. 5). In contrast, there

Table 3. Multiple Logistic Regression Analysis for AUC_{15} Values in Functional Dyspepsia Patients

Factors	OR (95% CI)	P-value
BMI (kg/m^2)	0.685 (0.522-0.899)	0.006[a]
Age (yr)	0.997 (0.956-1.041)	0.904
Leu72Met408 (GG, GT/TT)	15.06 (1.690-134.17)	0.015[a]
Gln90Leu3412 (TT, TA/AA)	0.982 (0.911-1.021)	0.994
Preproghrelin3056 (TT, TC/CC)	0.241 (0.036-1.621)	0.143
Arg51Gln346 (CC, CT/TT)	0.980 (0.908-1.124)	0.993
EPS-like[b]	1.055 (0.968-1.149)	0.224
PDS-like[c]	0.996 (0.924-1.073)	0.914

AUC_{15}, area under the curve at 15 minutes; BMI, body mass index; EPS, epigastric pain syndrome; PDS, postprandial distress syndrome.
[a]P-value of less than 0.05 was considered statistically significant.
[b]Most bothersome symptom based on physician interview was upper abdominal pain.
[c]Most bothersome symptom based on physician interview was early satiety.

was no significant relationship between the Gln90Leu3412 genotype and AUC_{15} value in healthy volunteers (Fig. 5).

Multiple Logistic Regression Analysis of AUC_{15} Value in Functional Dyspepsia Patients

To evaluate which factors are related to AUC_{15} value as a marker of early phase of gastric emptying, we investigated several parameters including BMI, age, Leu72Met408, Gln90Leu3412, preproghrelin3056, and Arg51Gln346 genotype SNPs, EPS-like symptoms, and PDS-like symptoms by multiple logistic regression analysis. BMI and the Leu72Met408 genotype were significantly associated with high levels of AUC_{15} values (> 41.17 [% dose], mean values + 2SD in healthy volunteers) in FD patients ($P = 0.006$ and $P = 0.015$, respectively) (Table 3). In contrast, other GHRL gene SNPs such as Gln90Leu3412, preproghrelin3056, and Arg51Gln346 were not significantly linked to AUC_{15} values in FD patients (Table 3). In our data, clinical symptoms including EPS-like symptoms and PDS-like symptoms were not significantly associated with AUC_{15} values in FD patients (Table 3).

Discussion

The major findings of this study are: (1) there was a significant relationship between the Gln90Leu genotype and SRQ-D score in FD patients; (2) AUC_5, AUC_{10}, and AUC_{15} values in FD patients were significantly greater compared to those in healthy volunteers; and (3) Leu72Met408 genotype SNP in FD patients was significantly associated with early phase of gastric emptying

by multiple logistic regression analysis.

In this study, we first reported that one of GHRL gene SNP, Gln90Leu genotype is significantly associated with SRQ-D score as a scale of depressive state in FD patients. In contrast, in healthy volunteers, the Gln90Leu genotype was not linked to SRQ-D score. This correlates with studies showing familial clustering of FD,[15] suggesting that a genetic factor may also play a significant role in the development of FD. In addition, the anxiogenic effect of GHRL was reported in a rat model[11] and an antidepressant-like effect of anti-sense DNA for ghrelin in rats may suggest a depressogenic effect.[31] However, in our previous study, the Gln90Leu genotype was not linked to acylated GHRL levels in *H. pylori*-negative FD patients.[22] In contrast, Ando et al[32] have reported that the Leu72Met408 genotype SNP is significantly associated with acylated GHRL levels. Further studies will be needed to clarify whether the Gln90Leu and the Leu72Met408 genotypes are linked to GHRL activity.

Brain-gut interaction modulates appetite, gut motility and digestion. Gastric motility is affected by various gut hormones such as motilin, GHRL, cholecystokinin, glucagon-like peptide-1 and peptide YY.[33] Tahara et al[34] have reported that a cholecystokinin genotype was associated with PDS in Japanese male subjects. In addition, we also previously reported a significant relationship between low levels of acylated GHR linked to appetite and T_{max} values.[4] However, several studies suggest that the relationship between plasma GHRL levels and FD remain uncertain.[4,35,36] In addition, Kusano et al[6] reported that early phase of gastric emptying is also contributed to FD patients as well as delayed gastric emptying.[31] Lee et al[30] reported that gastric flow into the duodenum including gastric acid inhibits gastric accommodation to a meal and contributes to postprandial symptom. Considering these reports, it warrants further exploration to clarify the precise mechanisms underlying impaired gastric emptying in early postprandial phase to truly understand the pathophysiology of FD. Therefore, in this study, we tried to determine whether there was a significant relationship between early phase of gastric emptying and GHRL genotypes in FD patients. We are the first to show that 2 GHRL genotypes, Leu72Met408 and Gln90-Leu3412, were significantly associated with early phase of gastric emptying. In this study, we investigated multiple logistic regression of high AUC_{15} value (> 41.7% dose, mean value + 2SD in healthy volunteers). The Leu72Met408 gene SNP was significantly linked to early phase of gastric emptying in this analysis. In contrast, other GHRL gene SNPs were not associated with early phase of gastric emptying. Entire gastric empty-

ing was not significantly associated with any of the four GHRL gene SNPs or with the GNβ3 gene SNP (Table 2). Ando et al[32] have reported that the Leu72Met408 genotype SNP was significantly associated with low levels of acylated GHRL. GHRL levels in the postprandial phase may also affect regulation of gastric motility and food intake, affecting secretion of glucagon-like peptide-1, an incretin hormone.[37,38] Considering our results and previous studies, dysregulation of early phase of gastric emptying, such as abnormality of AUC_{15} value, but not entire gastric emptying, may be associated with reduction of acylated GHRL levels in FD patients with the Leu72Met708GG genotype (dominant model). It may be of value to consider the pathophysiology of FD patients to determine the precise mechanisms involved in the disturbance of gastric motility in early postprandial phase. In this study, the Leu72Met408 gene SNP was not linked to early phase of gastric emptying in healthy volunteers. Further studies will be also needed to clarify why the Leu72Met408 genotype was associated with early phase of gastric emptying but not with entire gastric emptying in FD patients.

Analyses of dysregulation of early phase of gastric emptying are necessary to elucidate the pathophysiology of FD patients.[29] Accelerated gastric emptying in the early postprandial period may be associated with impaired accommodation.[39,40] In addition, in a scintigraphic study, early redistribution of the meal to the distal stomach or accelerated gastric emptying in the postprandial period, suggestive of impaired accommodation, was associated with symptoms of early satiety.[40] Gilja et al[41] have also reported that impaired accommodation was associated with meal-induced symptoms such as PDS-like symptoms. Although Leu72Met408 genotype in PDS patients (n = 42) was significantly ($P = 0.014$) associated with AUC_{15} value in our data, PDS-like symptoms were not significantly associated with gastric emptying in the early postprandial period in multiple logistic regression analysis. Further studies will be needed to clarify why there are certain discrepancies between other studies and our data. In addition, gastric emptying and duodenal glucose delivery are closely regulated.[42,43] Early phase of gastric emptying is usually 5-15 minutes in duration and is influenced by intragastric drink volume and associated with duodenal glucose delivery.[42,44,45] In this study, AUC_{15} value as a marker of early phase of gastric emptying was significantly associated with the Leu72Met408 gene SNP in FD patients. In addition, the Met72 allele has been shown to be associated with an earlier age of self-reported onset of obesity in several studies and with higher BMI values.[20] In our data, AUC_{15} value is also significantly linked to BMI in FD patients. The

Leu72Met genotype has also been reported to be associated with type 2 diabetes-related phenotypes.[46] Previous studies reported that non-insulin-dependent diabetes mellitus (NIDDM) patients exhibited accelerated gastric emptying.[47] Frank et al[48] also reported that accelerated gastric emptying in patients with non-neuropathic NIDDM is associated with increased proximal stomach phasic contractions. Therefore, the Leu72Met genotype might in part contribute to NIDDM patients through modification of early phase of gastric emptying. It may be important to consider the regulation of duodenal glucose delivery in evaluating early phase of gastric emptying using AUC_{15} value.

The present study has some limitations. In this study, we investigated Leu72Met 408 gene polymorphism in a limited region of Japan. In addition, because of the small number of subjects in the Rome III subgroups, type II error could not be excluded. Since Leu72Met408 gene polymorphism shows variations in different ethnic groups, additional studies will be needed in a larger and ethnically diverse population to evaluate the impact of Leu72Met408 gene polymorphism in FD patients. Another limitation of this study was that the precise physiological mechanism underlying disturbed gastric emptying in early phase of postprandial status in FD patients with the Leu72Met408 gene SNP remains unclear. Taken together, in this study, the GHRL gene genotype, the Leu72Met408 genotype SNP is significantly associated with early phase of gastric emptying in FD patients. Further studies are needed to clarify the mechanisms underlying the association between GHRL gene genotype SNPs and early phase of gastric emptying in FD patients.

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