J Neurogastroenterol Motil, Vol. 28 No. 1 January, 2022
pISSN: 2093-0879 eISSN: 2093-0887
https://doi.org/10.5056/jnm21047
Journal of Neurogastroenterology and Motility

Original Article

Sex and Gender Differences in Overlap Syndrome of Functional Gastrointestinal Disorder and Effect of Genetic Polymorphisms in South Korea: A Long-term Follow-up Study

Ju Yup Lee,[1,2] Nayoung Kim,[1,3]* Ji Hyun Park,[3] Jeong Eun Yu,[1] Yun Jeong Song,[1] Jung Won Yoon,[1] and Dong Ho Lee[1,3]

[1]Department of Internal Medicine, Seoul National University Bundang Hospital, Seoungnam, Gyeonggi-do, Korea; [2]Department of Internal Medicine, Keimyung University School of Medicine, Daegu, Korea; and [3]Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea

Background/Aims
Overlap functional gastrointestinal disorder (FGID) is associated with more severe gastrointestinal symptoms and lower quality of life. The aim of this study is to evaluate clinical features of non-erosive reflux disease (NERD), functional dyspepsia, irritable bowel syndrome, their overlap in terms of sex and gender, and to assess the risk factors, including genetic polymorphisms.

Methods
A total of 494 FGIDs and 239 controls were prospectively enrolled between 2004 and 2020. FGIDs were diagnosed based on the Rome III criteria and symptoms were evaluated using a questionnaire. Follow-up questionnaires were conducted to determine the change of symptoms during the 75.8-month mean observation period. Risk factors including genetic polymorphisms in neurotransmitter receptor (*SLC6A4 5-HTTLPR, GNB3, ADRA2A, CCKAR*, and *TRPV1*) and cytokine (*TNFA* and *IL10*) genes.

Results
NERD was more prevalent in men, and functional dyspepsia in women. Overlap FGIDs (n = 239) were more prevalent than non-overlap FGIDs (n = 255) in women ($P = 0.019$). Anxiety and depression scores were higher in the overlaps ($P = 0.012$ and $P < 0.001$, respectively). Symptoms were more frequent and severe in the overlap FGIDs than in the non-overlaps ($P < 0.001$). During follow-up, symptoms progressed more frequently in the overlap FGIDs, especially in patients with the L/S genotype of *SLC6A4 5-HTTLPR* and anxiety/depression.

Conclusions
Overlap FGID patients need attention given their association with anxiety/depression and more severe symptoms, especially in women. Genetic polymorphisms also may be associated with certain symptoms of overlap FGIDs.
(J Neurogastroenterol Motil 2022;28:145-158)

Key Words
Dyspepsia; Female; Male; Irritable bowel syndrome; Polymorphism

Received: February 23, 2021 Revised: October 2, 2021 Accepted: October 11, 2021

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Correspondence: Nayoung Kim, MD, PhD
Department of Internal Medicine, Seoul National University Bundang Hospital, 82, Gumi-ro 173beon-gil, Bundang-gu, Seongnam, Gyeonggi-do 13620, Korea
Tel: +82-31-787-7008, Fax: +82-31-787-4051, E-mail: nakim49@snu.ac.kr, nayoungkim49@empas.com

ⓒ 2022 The Korean Society of Neurogastroenterology and Motility

Introduction

Non-erosive reflux disease (NERD), functional dyspepsia (FD), and irritable bowel syndrome (IBS) are representative functional gastrointestinal disorders (FGIDs) without clinically detectable anatomical abnormalities, but elicit chronic and recurrent gastrointestinal (GI) symptoms.[1] Although these FGIDs may manifest alone, 2 or more overlapping cases are common and defined as an overlap syndrome.[2] Locke et al[3] found that 1-8% of the general population have reported 2 to 3 FGIDs, whereas Choung et al[4] found that 17% of the general population have reported more than 1 FGID. According to a systematic review and meta-analysis, the prevalence of IBS among FD patients was 37%, which was significantly higher than that among those without FD (7%).[5] In addition, the prevalence of FD in patients with IBS was 29-87%.[3,6,7]

FGID patients have a higher proportion of overlap syndrome than patients with other types of disorders which are not FGID,[8-10] suggesting that patients with overlap syndrome visit the hospital more frequently than those with FD or IBS alone. Patients with overlap syndrome also reported more frequent and severe GI symptoms, more severe depressive symptoms, and lower quality of life (QoL) than those with single FGID.[11] In actual clinical practice, overlap FGID can be frequently encountered, and it is important to know the clinical course of overlap FGID because these patients have severe symptoms and are highly likely to be related to psychosocial factors without responding to conservative treatment. However, there have been only few studies on the overlap syndrome[12-15] and on follow-up[16,17]; therefore, studies on the characteristics of patients with overlap syndrome and their associated risk factors are insufficient.

The various causes of FGIDs include changes in the motor and sensory nerves of the intestine, psycho-neurological factors, and changes in the immune system due to infection and allergy. Recently, gene polymorphisms affecting the pathogenesis of FGIDs in families or twins were discovered.[18] Previously, we suggested that genetic polymorphisms in the serotonin transporter gene-linked long polymorphic region (*SLC6A4* 5-HTTLPR) and the alpha 2A adrenergic receptor (*ADRA2A*) 1291C>G polymorphism are pathological factors of IBS.[19] We also identified *SLC6A4* 5-HTTLPR and the 945G>C polymorphism in transient receptor potential ion channel of the vanilloid type 1 (*TRPV1*) among Korean FD patients, particularly those positive for *Helicobacter pylori* infection.[20] In addition, 5-HTTLPR L/L has been found to be a risk factor for the co-occurrence of IBS with constipation and FD.[21]

Moreover, in NERD patients, sensitivity to stress often determines the expression of symptoms; thus, NERD has been classified as an FGID and hypothesized to have an etiology different from that of reflux esophagitis. NERD represents the more common phenotypic presentation of GERD and comprises of patients who have typical symptoms without any mucosal breaks at endoscopy. However, these patients are markedly heterogeneous from a pathophysiological point of view.[22] These groups consist of patients with esophageal acid exposure (true NERD), normal esophageal acid exposure but a positive symptom association probability for acid reflux (acid hypersensitive esophagus), or with normal esophageal acid exposure and a negative symptom association probability for any type of reflux (functional heartburn).[22,23] Recently, the Rome criteria (Rome IV) for functional esophageal disorders, in which acid hypersensitive esophagus and non-acid hypersensitive esophagus groups have been combined into a new category of reflux hypersensitivity (RH) that would appear to fit somewhere between NERD and functional heartburn, has been defined.[23,24] NERD, FD, and IBS often develop together in siblings, especially in families that carry a common genetic polymorphism. However, as the diagnosis of FGID depends on the patient's subjective symptoms rather than clinical objective findings, and its etiology is often complex, studies focusing on the genes mentioned above are few, and investigation of genes in combination is rare.

Based on this background information, we hypothesized that the symptoms in overlap FGIDs may be severe and more progressive, and polymorphisms in genes involved in neurotransmission and cytokines may contribute to this overlap in FGIDs compared with non-overlap FGIDs. The aim of this study is to analyze the prevalence of overlap syndromes among NERD, FD, and IBS patients, to elucidate the differences in their characteristics and symptoms, and to determine their risk factors according to their sex and gender. In addition, we aim to analyze the effect of genetic polymorphisms and anxiety/depression on FGIDs depending on whether they are non-overlap or overlap over a long follow-up period.

Materials and Methods

Study Subjects

Korean subjects were prospectively enrolled at the gastroenterology outpatient clinic of the Seoul National University Bundang Hospital (SNUBH) between July 2004 and August 2020. The inclusion criteria were as follows: provision of consent to participate in the study, aged 18-80 years, and scheduled upper endoscopy

procedure. Colonoscopy and abdominal imaging studies (eg, abdominal ultrasound or abdominopelvic computed tomography) were performed with upper endoscopy in the presence of clinical indications. Healthy control subjects and patients with FGID were collected as follows: subjects who underwent GI evaluations as a health check-up or for other problems based on a family history of gastric or colon cancer were categorized as healthy controls, whereas those who visited the GI clinic for the evaluation of upper or lower GI symptoms were classified as cases upon consenting to participate in this study. This classification of healthy controls and cases was finally revised in the analysis step of the questionnaire survey. Exclusion criteria were as follows: history of GI surgery except appendectomy, inflammatory bowel disease, any malignancy, or systemic diseases requiring chronic medication except for hypertension and diabetes mellitus; pregnant or lactating women; and patients with hepatic, biliary, or psychiatric disorders requiring medication. Subjects were excluded from the study if organic diseases were found during follow-up endoscopy. The Institutional Review Board of SNUBH approved this study (B-2006-616-128), and written informed consent was obtained from all participants. This study protocol has been registered at ClinicalTrials.gov (NCT04712617).

Diagnostic Questionnaire of Functional Gastrointestinal Disorders and Demographic Data

After enrollment, the subjects received GI endoscopy regularly and were requested to fill up the Korean Bowel Disease Questionnaire (K-BDQ)[19,20] and gastroesophageal reflux questionnaire[25-28] which included 7 reflux symptom such as heartburn, acid regurgitation, chest pain, hoarseness, globus sensation, cough, and epigastric soreness whenever they undertook GI endoscopy.

NERD was defined as a typical heartburn occurring at least once a week without visible esophageal mucosal break, detected using endoscopy.[29] Subjects with minimal changes on endoscopy and with typical reflux symptoms were also classified as NERD.[30] A Korean Rome III criteria-based questionnaire translated from the original K-BDQ containing questions on 56 GI-related symptoms, sociodemographic status, medical history, smoking, alcohol habits, marital status, educational level, and employment status was prepared and validated.[19,20,31]

FD was diagnosed based on at least 1 of the following symptoms: bothersome postprandial fullness, early satiation, epigastric pain, and epigastric burning.[32] Absence of any structural diseases (including on upper endoscopy) that are likely to explain the symptoms was required. FD was categorized as postprandial distress syndrome, epigastric pain syndrome, or mixed subtype based on the Rome III criteria.[32,33] Postprandial distress syndrome was defined as the presence of meal-induced dyspeptic symptoms such as postprandial fullness and early satiation. Epigastric pain syndrome was diagnosed based on the presence of epigastric pain or burning sensations.[32,33]

IBS was defined by recurrent abdominal pain or discomfort with at least 2 of the following characteristics: relief with defecation, onset associated with a change in frequency of stool, and onset associated with a change in form (appearance) of stool.[34] IBS was subclassified into IBS with constipation, IBS with diarrhea, mixed IBS, or unsubtyped IBS, as described previously.[21]

Assessment of Functional Gastrointestinal Disorders Symptoms, Anxiety, Depression, and Quality of Life

Representative GI symptoms were classified according to frequency and severity. Symptom severity was calculated by adding the severity scores (from 0 to 4: 0, none; 1, mild; 2, moderate; 3, severe; and 4, very severe) using the K-BDQ.[35] Stool consistency based on the Bristol Stool Form Scale[36] and number of bowel habits were also evaluated.

Anxiety and depression were assessed using the Hospital Anxiety and Depression Scale (HADS).[37] They were subdivided into anxiety and depression subscales, both containing 7 items each. Each response is ranked on a scale from 0 to 3. A higher HADS score indicates that the subject is more depressive or anxious. A total score of 8 or more for each subscale indicates potential anxiety disorder or depression.[38]

Genotyping

Genomic DNA from blood samples was isolated using QIAamp DNA blood mini kit (QIAGEN Inc, Valencia, CA, USA) following the manufacturer's instructions, as reported previously.[19] To detect the presence of the long (L) or short (S) allele of the *SLC6A4* gene (5-HTTLPR), polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) assay was performed on a Perkin Elmer real time PCR machine model 9600 (Perkin Elmer, Norwalk, CT, USA) with the forward primer 5'-TCCTCCGCTTTGGCGCCTCTTCC-3' and reverse primer 5'-TGGGGGTTGCAGGGGAGATCCTG-3'. The 469-bp fragment of the 5-HTTLPR polymorphism was designated "S," and the 512-bp fragment was designated "L." The genotyping of the CCK1R intron-779T>C polymorphism was also performed using the PCR-RFLP assay with the forward primer 5'-CTGTTCACTTGAGGAGCTTTG-3' and the reverse primer 5'-TTAGAAGCTGACCTCCAACATGG-3'.

The PCR product was digested using PstI; the T allele yielded DNA fragments of 264/480 bp, whereas the C allele, 744 bp. The G-protein β3 (*GNB3*) 825C>T, *ADRA2A*−1291C>G, cholecystokinin receptor 1 (*CCK1R*) intron 779T>C, *TRPV1* 945G>C, tumor necrosis factor-α (*TNFA*) 308 G>A, and interleukin (*IL*) *10* 592C>A single nucleotide polymorphisms were determined using 5-exonuclease TaqMan genotyping assays on an ABI StepOnePlus Real-Time PCR System, according to the

Table 1. Demographic and Baseline Characteristics

Variables	Healthy control (n = 239)	NERD only (n = 116)	FD only (n = 90)	IBS only (n = 49)	NERD-FD (n = 108)	NERD-IBS (n = 21)	FD-IBS (n = 51)	NERD-FD-IBS (n = 59)
Age (yr)	55.1 ± 11.7	57.0 ± 12.2	52.5 ± 12.7	46.8 ± 15.2[a]	51.3 ± 13.1	50.5 ± 10.7	50.9 ± 13.6	44.0 ± 13.2[a]
Women	146 (61.1)	44 (37.9)[a]	68 (75.6)[a]	20 (40.8)	68 (63.0)	11 (52.4)	35 (68.8)	38 (64.4)
Body mass index (kg/m^2)	23.4 ± 3.4	24.1 ± 3.1	22.2 ± 3.3	22.7 ± 2.9	22.7 ± 3.1	23.3 ± 2.8	22.6 ± 3.6	23.0 ± 3.4
Marriage								
Single/divorced/widowed	32 (13.7)	15 (12.9)	8 (8.9)	6 (14.0)	18 (16.7)	2 (9.5)	13 (25.5)[a]	21 (35.6)[a]
Married	201 (86.3)	101 (87.1)	82 (91.1)	37 (86.0)	90 (83.3)	19 (90.5)	38 (74.5)	38 (64.4)
Education								
Elementary/middle school	34 (16.3)	16 (17.6)	13 (16.7)	1 (2.9)	13 (14.1)	4 (20.0)	9 (23.7)	10 (19.2)
High school	44 (21.2)	25 (27.5)	30 (38.5)	4 (11.4)	36 (39.2)	4 (20.0)	9 (23.7)	14 (26.9)
University	130 (62.5)	50 (54.9)	35 (44.9)	30 (85.7)	43 (46.7)	12 (60.0)	20 (52.6)	28 (53.8)
Job								
Employed	109 (52.9)	60 (67.4)	31 (39.7)	23 (65.7)	46 (50.5)	15 (75.0)	24 (57.2)	27 (54.0)
Unemployed	22 (10.7)	10 (11.3)	6 (7.7)	3 (8.6)	7 (7.7)	0 (0.0)	3 (7.1)	4 (8.0)
Housewives	75 (36.4)	19 (21.3)	41 (52.6)	9 (25.7)	38 (41.8)	5 (25.0)	15 (35.7)	19 (38.0)
Alcohol (> 35 g/wk)	29 (12.4)	21 (18.1)	4 (4.4)	6 (24.0)	10 (9.3)	1 (4.8)	12 (23.5)	9 (15.3)
Current smoker	11 (4.7)	19 (16.4)	9 (10.0)	6 (24.0)	15 (13.9)	3 (14.3)	7 (13.7)	10 (16.9)
Daily spicy food ingestion	160 (77.7)	73 (73.0)	55 (69.6)	27 (75.0)	73 (75.3)	15 (78.9)	26 (65.0)	40 (76.9)
Anxiety HADS score	5.1 ± 3.5	4.6 ± 2.7	7.9 ± 5.6	6.0 ± 3.3	6.1 ± 3.6	6.7 ± 3.1	8.0 ± 3.6	9.0 ± 3.7[b]
Depression HADS score	5.3 ± 3.5	5.3 ± 3.2	6.9 ± 2.9	6.0 ± 3.3	7.3 ± 3.4	9.7 ± 1.2	10.0 ± 3.6	10.2 ± 3.6[a]

[a]$P < 0.05$ compared to control.
[b]$P < 0.05$ compared to NERD only group and control.
NERD, non-erosive reflux disease; FD, functional dyspepsia; IBS, irritable bowel syndrome; HADS, hospital anxiety and depression scale.
Data are expressed as mean ± SD or n (%).

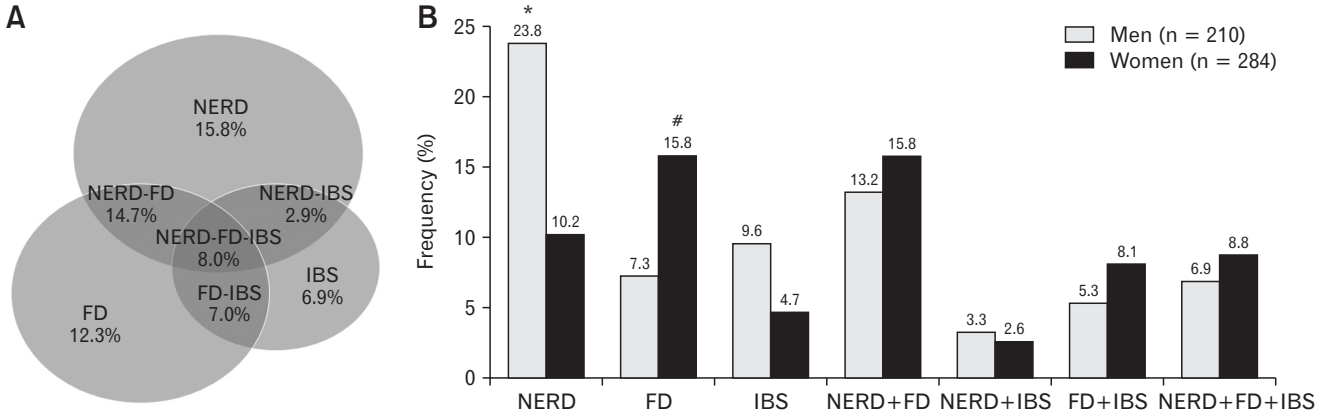

Figure 1. Proportions of subjects with functional gastrointestinal disorders (FGIDs) (A) and distribution of FGIDs according to sex (B). The prevalence of non-erosive reflux disease (NERD) was significantly higher in men and that of functional dyspepsia (FD) was significantly higher in women. IBS, irritable bowel syndrome. *$P < 0.001$ compared with women NERD, #$P < 0.001$ compared with men FD. FGID (n = 494), NERD (n = 304), FD (n = 308), irritable bowel syndrome (IBS) (n = 180), and non-overlap FGIDs (n = 255), overlap FGIDs (n = 239).

manufacturer's instructions (Applied Biosystems, Foster City, CA, USA).[20,21] The predesigned primer and probe sets were ordered at http://www. appliedbiosystems.com (*GNB3* 825C>T assay ID number: C___2184734_10; *ADRA2A*−1291C>G assay ID number: C___7611979_10; *TRPV1* 945G>C assay ID number: C___1093688_20) and used according to their protocols. The genotyping results of *SLC6A4* 5-HTTLPR, *GNB3* 825C>T, *ADRA2A*−1291C>G, *CCK1R* intron 779T>C, *TRPV1* 945G>C, *TNFA308* G>A, and *IL10* 592C>A were confirmed using direct sequencing, with the ABI version 3.1 Sequence Analysis software (Applied Biosystems).

Statistical Methods

Data are expressed as mean (standard deviation or standard error), geometric mean (95% CI), or percentage. Comparisons among 3 or more groups were performed using the chi-square test in categorical variables or analysis of variance (ANOVA) followed by Bonferroni correction in continuous variables. Two-way repeated measures ANOVA was performed to determine whether the symptom scores were different within a group and between groups at baseline, and at first and second follow-up time points. A post-hoc Tukey's test was used to identify the group that showed a difference when ANOVA showed a significant interaction. Variables with $P < 0.05$ in univariate analyses or clinical importance were subjected to multivariate analyses. All statistical tests were 2-tailed. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the SPSS 21.0 statistical package (version 21.0, IBM, Armonk, NY, USA).

Results

Demographic and Baseline Characteristics

A total of 733 subjects participated in this study (Table 1). The mean age was 52.7 ± 13.1 years, and 430 (58.7%) subjects were women. A total of 494 subjects were diagnosed with NERD, FD,

Table 2. Clinical Characteristics of Non-overlap and Overlap Functional Gastrointestinal Disorders

Variables	Healthy control (a) (n = 239)	Non-overlap FGID (b) (n = 255)	Overlap FGID (c) (n = 239)	*P*-value	Post-hoc
Age (yr)	55.1 ± 11.7	53.5 ± 13.5	49.4 ± 13.3	< 0.001	a > b, a > c
Females	146 (61.1)	132 (51.8)	152 (63.6)	0.019	a > b, b < c
Body mass index (kg/m^2)	23.4 ± 3.4	23.2 ± 3.2	22.8 ± 3.2	0.139	
Marriage				0.002	a < c, b < c
Single/divorced/widowed	32 (13.7)	29 (11.6)	54 (22.6)		
Married	201 (86.3)	220 (88.4)	185 (77.4)		
Education				0.125	
Elementary/middle school	34 (16.3)	30 (14.7)	59 (28.9)		
High school	44 (21.2)	115 (56.4)	35 (17.8)		
University	130 (62.5)	63 (31.2)	103 (51.0)		
Job				0.677	
Employed	109 (52.9)	114 (56.4)	19 (9.4)		
Unemployed	22 (10.7)	69 (34.2)	112 (55.2)		
Housewives	75 (36.4)	14 (6.9)	77 (37.9)		
Alcohol (> 35 g/wk)	29 (12.4)	31 (13.4)	32 (13.4)	0.939	
Current smoker	11 (4.7)	34 (14.7)	35 (14.6)	< 0.001	a < b, a < c
Daily spicy food ingestion	160 (77.7)	155 (72.1)	154 (74.0)	0.413	
Anxiety HADS score	5.1 ± 3.5	5.2 ± 3.3	6.2 ± 3.7	0.012	a < c, b < c
Men	5.2 ± 3.0	5.1 ± 2.9	5.7 ± 2.9	0.614	
Women	5.0 ± 3.9	5.3 ± 4.2	8.3 ± 4.1[a]	0.011	a < c, b < c
Depression HADS score	5.3 ± 3.5	5.5 ± 3.2	8.2 ± 3.6	< 0.001	a < c, b < c
Men	4.9 ± 2.7	5.5 ± 3.2	7.4 ± 3.2	0.007	a < c, b < c
Women	5.7 ± 4.1	5.6 ± 3.3	9.2 ± 3.9[b]	0.001	a < c, b < c

[a]$P = 0.007$ compared to men.
[b]$P = 0.056$ compared to men.
FGID, functional gastrointestinal disorder; HADS, hospital anxiety and depression scale.
Data are expressed as mean ± SD or n (%).

and/or IBS, including 14.7% NERD-FD, 2.9% NERD-IBS, 7.0% IBS-FD, and 8.0% NERD-FD-IBS 3 overlap; 239 subjects were identified as healthy controls (Fig. 1A). The prevalence of NERD was significantly higher in men, whereas that of FD was significantly higher in women (Fig. 1B). The age of occurrence in the NERD-FD-IBS group was significantly lower than that in the control group ($P < 0.05$) (Table 1). The proportions of single, divorced, and widowed patients were significantly higher in the FD-IBS ($P < 0.05$) and NERD-FD-IBS ($P < 0.05$) groups than in the control group. Anxiety and depression (HADS) scores were also significantly higher in the NERD-FD-IBS group ($P < 0.05$) than those in the control group (Table 1).

Clinical Characteristics of Non-overlap and Overlap Functional Gastrointestinal Disorders

The number of non-overlap and overlap FGID subjects was 255 and 239, respectively. The average age of the patients in the overlap FGID group was lower than that of the non-overlap group ($P < 0.001$). The percentage of women in the overlap FGID group was higher than that in the non-overlap FGID group ($P = 0.019$), and the percentages of single, divorced, and widowed patients were higher than those in the healthy controls ($P = 0.018$) and non-overlap FGID group ($P = 0.002$) (Table 2). Anxiety and depression scores were also higher in the overlap FGID group than in the healthy control and non-overlap FGID groups ($P = 0.012$ in anxiety score and $P < 0.001$, respectively). Anxiety and depression scores were higher in women in the FGID group ($P = 0.007$ for anxiety and $P = 0.056$ for depression) (Table 2).

Frequency and Severity of Gastrointestinal Symptoms Between Non-overlap and Overlap Functional Gastrointestinal Disorders

All upper and lower GI symptoms were significantly more frequent in the overlap FGID group than in the non-overlap FGID group and healthy control group. The severity of upper GI symptoms was significantly higher in the overlap FGID group than in the non-overlap FGID group ($P < 0.001$) (Table 3).

Genetic Polymorphisms

The G allele and G/G genotype of *ADRA2A* were significantly associated with NERD-FD in both sexes ($P = 0.038$ in total, $P = 0.048$ in men, and $P = 0.046$ in women). The T allele of *GNB3* 825C>T was significantly associated with IBS ($P = 0.035$), but there were no gender-based differences. The T allele of

Table 3. Frequency and Severity of Gastrointestinal Symptoms Between Non-overlap and Overlap Functional Gastrointestinal Disorders

Variables	Healthy control (a) (n = 239)	Non-overlap FGID (b) (n = 255)	Overlap FGID (c) (n = 239)	*P*-value	Post-hoc
Upper GI symptoms frequency					
Early satiation	26 (11.2)	51 (20.6)	136 (57.1)	< 0.001	a < b < c
Postprandial fullness	30 (12.9)	83 (33.6)	185 (77.4)	< 0.001	a < b < c
Epigastric pain/burning	40 (17.3)	88 (35.5)	169 (71.3)	< 0.001	a < b < c
Bloating	31 (13.7)	65 (26.9)	146 (63.2)	< 0.001	a < b < c
Nausea	19 (8.3)	31 (13.1)	101 (43.2)	< 0.001	a < b < c
Vomiting	7 (3.1)	10 (4.2)	37 (15.7)	< 0.001	a < b < c
Upper GI symptoms severity (5 Likert score [0-4])					
Early satiation symptom severity	0.0 ± 0.1	0.5 ± 0.1	1.8 ± 0.1	< 0.001	a < b < c
Postprandial fullness symptom severity	0.0 ± 0.1	0.8 ± 0.1	2.5 ± 0.1	< 0.001	a < b < c
Epigastric pain symptom severity	0.3 ± 0.1	1.0 ± 0.1	2.2 ± 0.1	< 0.001	a < b < c
Bloating pain symptom severity	0.3 ± 0.1	0.7 ± 0.1	2.0 ± 0.1	< 0.001	a < b < c
Lower GI symptoms frequency					
Less than 3 bowel movements/wk	18 (8.0)	21 (8.8)	23 (9.8)	0.803	
Hard or lumpy stools	70 (30.0)	93 (37.5)	146 (61.1)	< 0.001	a < b < c
Loose or watery stools	54 (23.6)	92 (37.2)	137 (57.6)	< 0.001	a < b < c
Defecation straining	53 (23.3)	91 (37.4)	144 (60.3)	< 0.001	a < b < c
A feeling of incomplete bowel movement	51 (43.2)	99 (84.6)	158 (94.6)	< 0.001	a < b < c
Urgency	8 (3.5)	23 (9.6)	26 (11.1)	0.007	a < b < c

GI, gastrointestinal; FGID, functional gastrointestinal disorder.
Data are expressed as n (%) or mean ± SE.

GNB3 825C>T was also significantly associated with increased susceptibility to NERD-FD-IBS overlap in both men ($P = 0.031$) and women ($P = 0.044$). The *SLC6A4* 5-HTTLPR L/L genotype and S allele ($P = 0.049$) and the *SCL6A4* 3609A>G G allele ($P = 0.001$) were associated with NERD-FD-IBS overlap, especially in women. The A allele of IL10 592C>A was associated with IBS in men ($P = 0.009$) (Table 4).

Table 4. Distribution of Genetic Polymorphisms Between Control and Functional Gastrointestinal Disorders

Genotypes	Controls	FGID					
		Total	*P*-value	Men	*P*-value[a]	Women	*P*-value[b]
SLC6A4 5-HTTLPR		NERD-FD-IBS					
S/S	92 (70.2)	27 (54.0)	0.049	12 (63.2)	0.681	15 (48.4)	0.024
L/S	35 (26.7)	18 (36.0)		7 (36.8)		11 (35.5)	
L/L	4 (3.1)	5 (10.0)		0 (0.0)		5 (16.1)	
SLC6A4 3609A>G		NERD-FD-IBS					
A/A	187 (89.5)	41 (75.9)	0.001	15 (75.0)	0.147	26 (76.5)	0.028
A/G	22 (10.5)	12 (22.2)		5 (25.0)		7 (20.6)	
G/G	0 (0.0)	1 (1.9)		0 (0.0)		1 (2.9)	
GNB3 825C>T		IBS only					
C/C	50 (23.8)	5 (12.8)	0.035	2 (9.5)	0.269	3 (16.7)	0.181
C/T	98 (46.7)	27 (69.3)		15 (71.4)		12 (66.7)	
T/T	62 (29.5)	7 (17.9)		4 (19.0)		3 (16.7)	
GNB3 825C>T		NERD-FD-IBS					
C/C	50 (23.8)	21 (38.9)	0.043	9 (45.0)	0.031	12 (35.3)	0.044
C/T	98 (46.7)	24 (44.4)		5 (25.0)		19 (55.9)	
T/T	62 (29.5)	9 (16.7)		6 (30.0)		3 (8.8)	
ADRA2A 1291C>G		NERD-FD					
C/C	21 (9.9)	19 (18.6)	0.038	9 (24.3)	0.048	10 (15.4)	0.046
C/G	98 (46.0)	35 (34.3)		16 (43.2)		19 (29.2)	
G/G	94 (44.1)	48 (47.1)		12 (32.4)		36 (55.4)	
CCKAR intron 779T>C		NERD-FD-IBS					
T/T	113 (51.9)	24 (44.4)	0.429	11 (55.0)	0.234	13 (38.2)	0.456
T/C	84 (38.5)	26 (48.1)		9 (45.0)		17 (50.0)	
C/C	21 (9.6)	4 (7.4)		0 (0.0)		4 (11.8)	
TRPV1 945G>C		NERD-FD-IBS					
G/G	52 (24.9)	13 (24.5)	0.295	4 (20.0)	0.577	9 (27.3)	0.393
G/C	97 (46.4)	30 (56.6)		11 (55.0)		19 (57.6)	
C/C	60 (28.7)	10 (18.9)		5 (25.0)		5 (15.2)	
TNFA 308G>A		NERD-FD-IBS					
G/G	194 (93.3)	24 (80.0)	0.032	11 (78.6)	0.254	13 (81.3)	0.144
G/A	13 (6.3)	6 (20.0)		3 (21.4)		3 (18.8)	
A/A	1 (0.4)	0 (0.0)		0 (0.0)		0 (0.0)	
IL10 592C>A		IBS only					
C/C	29 (13.9)	0 (0.0)	0.047	0 (0.0)	0.009	0 (0.0)	0.653
C/A	86 (41.1)	8 (80.0)		7 (100.0)		1 (33.3)	
A/A	94 (45.0)	2 (20.0)		0 (0.0)		2 (66.7)	

[a]*P*-values compared to men controls.
[b]*P*-values compared to women controls.
FGID, functional gastrointestinal disorder; NERD, non-erosive reflux disease; FD, functional dyspepsia; IBS, irritable bowel syndrome; 5-HTTLPR, serotonin transporter long polymorphic region; *GNB3*, G-protein β3 gene; *ADRA2A*, alpha 2A adrenergic receptor gene; TRPV1, transient receptor potential ion channel of the vanilloid type 1; *CCK1R*, cholecystokinin receptor 1 gene; *TNFA*, tumor necrosis factor-α gene; *IL*, interleukin gene.
Data are expressed as n (%).

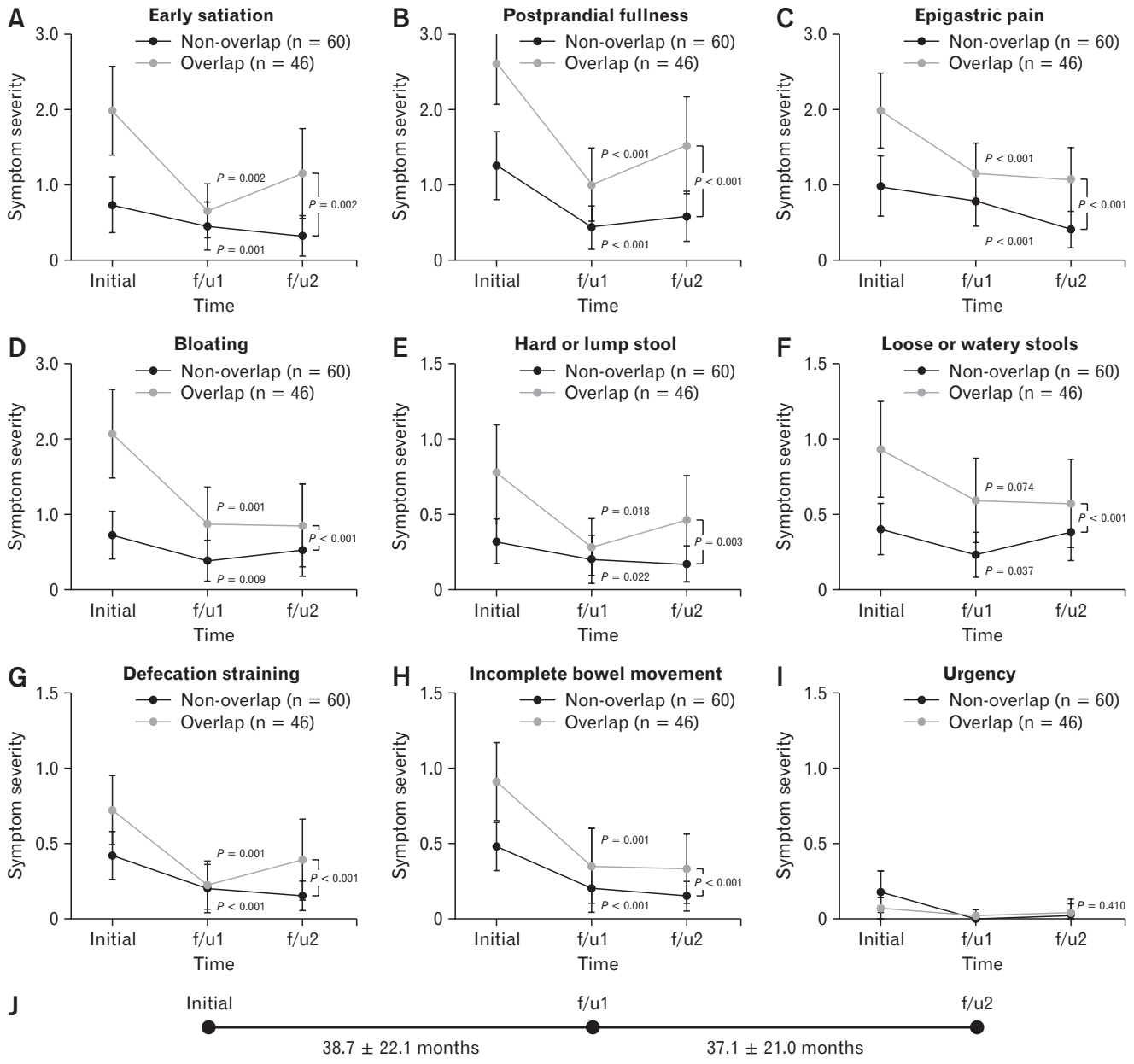

Figure 2. Changes in functional gastrointestinal disorder (FGID) symptoms during the follow-up period. Most FGID symptoms such as epigastric pain (C), bloating (D), loose or watery stool (F), incomplete defecation (H) and urgency (I) improved; however, symptoms of early satiation (A), postprandial fullness (B), hard or lump stool (E), and defecation straining (G) were aggravated in the overlap FGID group in the second follow-up visit. Symptoms were analyzed in the follow-up 1 (f/u1) (38.7 ± 22.1 months) and follow-up 2 (f/u2) 37.1 ± 21.0 months (J) periods.

Changes in Functional Gastrointestinal Disorder Symptoms From Baseline to Long-term Follow-up

After the initial baseline questionnaire, a follow-up questionnaire (n = 106) was used to determine the occurrence of 4 upper and 5 lower GI symptoms during the observation period. The mean period from baseline to the first follow-up (f/u1) was 38.7 ± 22.1 months, and that from the first follow-up to the second follow-up (f/u2) was 37.1 ± 21.0 months (Fig. 2J). Except for stool urgency, the intensity of all GI symptoms was more severe in the overlap FGID group than in the non-overlap FGID group, even during the follow-up period. During follow-up of the overlap FGID group, most FGID symptoms improved, such as epigastric pain (Fig. 2C), bloating (Fig. 2D), loose or watery stools (Fig. 2F),

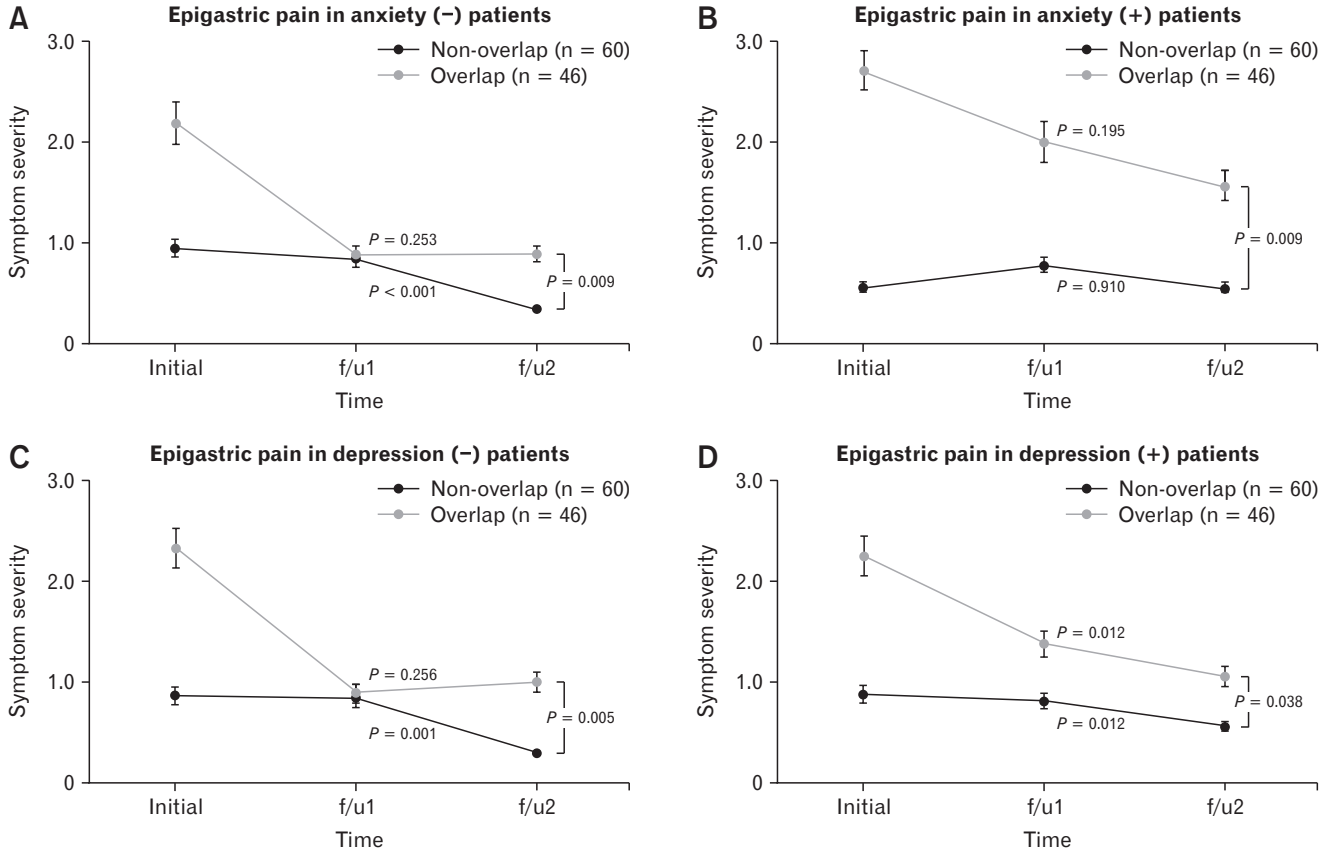

Figure 3. Changes in epigastric pain symptom severity according to anxiety and depression during the follow-up period. There were no differences in symptoms between the non-overlap and overlap functional gastrointestinal disorder (FGID) in anxiety (−) and depression (−) groups (A and C), however, differences between the non-overlap and overlap FGID were revealed during the follow-up period in anxiety (+) and depression (+) groups (B and D). There was no change in symptom severity in both non-overlap and overlap FGIDs during the follow-up period in the anxiety group (B). (+), patients has anxiety or depression; (−), patients has no anxiety or depression; f/u, follow-up.

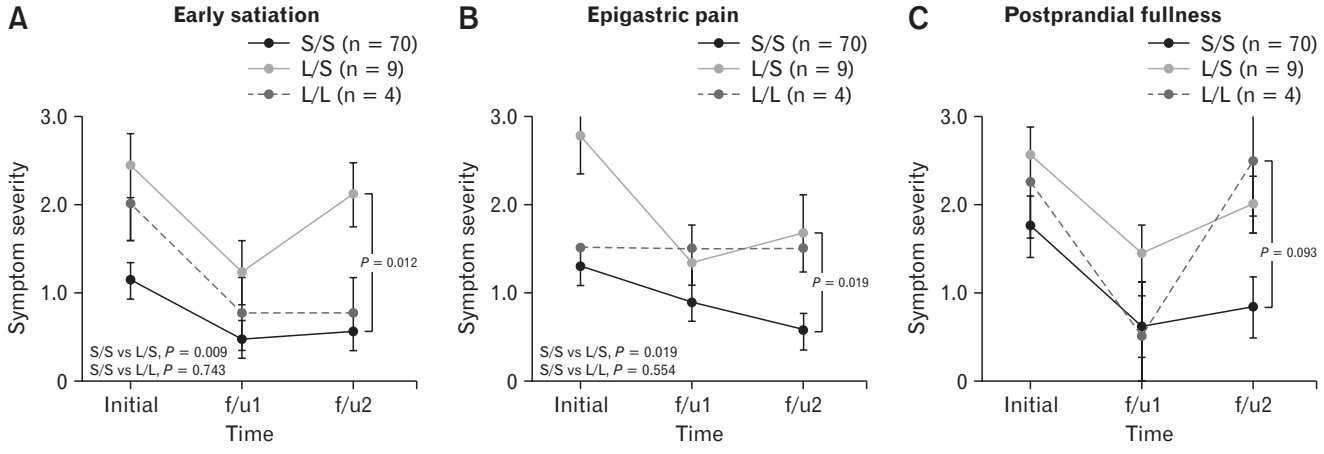

Figure 4. Changes in severity of upper gastrointestinal symptoms during the follow-up period according to the serotonin transporter gene-linked long polymorphic region (*SLC6A4* 5-HTTLPR) gene polymorphism. Early satiation (A) and postprandial fullness (C) were most severe in patients with the *SLC6A4* 5-HTTLPR L/S genotype and were found to worsen. Epigastric pain symptoms (B) in patients with the *SLC6A4* 5-HTTLPR L/L genotype were maintained and did not deteriorate or improve. L, long allele of the *SLC6A4* 5-HTTLPR gene; S, short allele of the *SLC6A4* 5-HTTLPR gene; f/u, follow-up.

incomplete defecation (Fig. 2H), and urgency (Fig. 2I); however, symptoms of early satiation (Fig. 2A), postprandial fullness (Fig. 2B), hard or lump stool (Fig. 2E), and defecation straining (Fig. 2G) were aggravated.

Among the various symptoms of FGID, epigastric pain was associated with anxiety and depression (Fig. 3). For patients with anxiety and depression, differences in symptoms severity between the non-overlap and overlap FGID groups were clearly revealed at initial, follow-up 1, and follow-up 2 ($P = 0.009$ for anxiety and $P = 0.038$ for depression) (Fig. 3B and 3D). In addition, there was no change in symptom severity in both non-overlap and overlap FGIDs during the follow-up period in the anxiety group (Fig. 3B).

A multivariate analysis was performed to determine whether genetic polymorphisms affect the development of symptoms. Early satiation (Fig. 4A) and postprandial fullness (Fig. 4C) were most severe in patients with the *SLC6A4* 5-HTTLPR L/S genotype and worsened during follow-up. In addition, the epigastric pain symptoms in patients with the *SLC6A4* 5-HTTLPR L/L genotype were maintained and did not deteriorate or improve during the follow-up period (Fig 4B). Multivariate analysis was performed to determine the relationship between the genetic polymorphism and overlap syndrome. Early satiation and epigastric pain symptoms in the non-overlap FGID group were not related to the *SLC6A4* 5-HTTLPR genotype. However, in the overlap FGID group, the L/L and L/S genotypes showed more severe symptoms than the S/S genotype (Fig. 5).

Discussion

In this study, we found sex/gender differences in FGID, especially in the overlap group. NERD was significantly more prevalent in men and FD was more prevalent in women. Overlap FGIDs were more prevalent than non-overlap FGIDs in women. Anxiety and depression scores were higher in the overlap FGIDs and

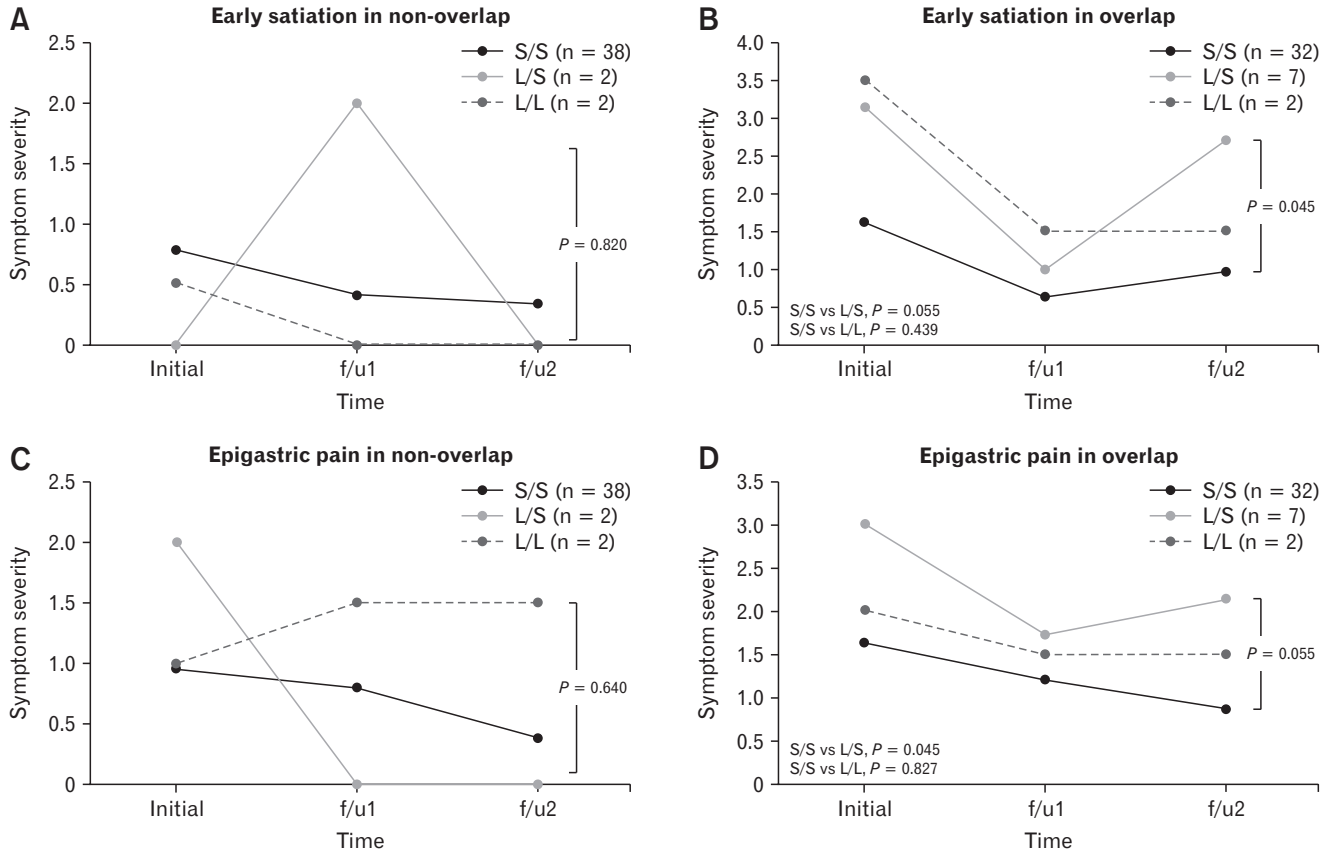

Figure 5. Effect of the serotonin transporter gene-linked long polymorphic region (*SLC6A4* 5-HTTLPR) gene polymorphism on the severity of epigastric pain in patients with overlap syndrome. Early satiation and epigastric pain symptoms in the non-overlap functional gastrointestinal disorder (FGID) group were not related to the *SLC6A4* 5-HTTLPR genotype (A, C). In the overlap FGID group, L/L and L/S genotypes elicited more severe symptoms than the S/S genotype (B, D). L, long allele of the *SLC6A4* 5-HTTLPR gene; S, short allele of the *SLC6A4* 5-HTTLPR gene; f/u, follow-up.

FGID symptoms (early satiation, postprandial fullness, and epigastric pain) were more frequent and severe in the overlap FGIDs than in the non-overlap groups. Symptom progress during long-term follow-up was more frequently observed in the overlap FGIDs, especially the L/S genotype of *SLC6A4 5-HTTLPR*. Polymorphism of *IL10* 592C>A was associated with the occurrence of IBS in men, and *SLC6A4 5-HTTLPR* was associated with female NERD-FD-IBS.

In a previous study on the general Korean population, the dyspepsia-IBS overlap was the most common and overlap FGID developed at a younger age and was more prevalent in women than non-overlapping FGIDs.[39] In another study that analyzed the relationship between FD, IBS, and reflux esophagitis patients, the odds ratio of having both FD and IBS was estimated to be 4.4 (95% CI, 1.21-15.71). However, there was no significant association between age, gender in patients with overlap syndrome.[40] Lee et al[11] found that, in psychiatric patients, the NERD-FD overlap was the most common among NERD, FD, IBS, and functional constipation.

Moreover, in our study, anxiety and depression scores were higher in the overlap FGID group. The frequency and severity score of various symptoms of FGIDs were higher in the overlap FGID group than in the control and non-overlapping FGID groups. Patients with both FD and IBS have been shown to experience more severe symptoms and worse QoL.[41-43] In another study, Korean patients with FD-IBS overlap were frequently found to be depressed and had a poor QoL.[43] Therefore, the authors of that study suggested that patients with FD-IBS overlap should be treated for their gastrointestinal symptoms and receive psychiatric support to improve their QoL and psychological distress.[43]

Psychosocial factors, including stress, play an important role in the expression of FGID.[44] In addition, when FGID is associated with mental illness such as depression or anxiety, the symptoms will be much worse.[45] In a previous study, the prevalence of depressive symptoms and anxiety in IBS patients were very high, at 22% and 30%, respectively.[46] Pinto-Sanchez et al[47] reported that, within the FGID group, a greater number of FGIDs was associated with a proportional increase in depression and anxiety. Recent animal studies have proposed a brain-gut-microbiome axis, in which stress changes the composition of the microbiome, and the microbiota, in turn, alter metabolism and transmission of brain-derived neurotrophic factor, gamma aminobutyric acid, and serotonin, and this influences depression and anxiety.[48] Vanuytel et al[49] reported that increased corticotrophin-releasing factor in the event of acute emotional stress alters intestinal permeability via the action of mast cells, and that corticotrophin-releasing factor and mast cells also affect visceral sensitivity.[50]

Very few studies have been conducted on the long-term follow-up of the overlap syndrome. Similar to our results, as in most observational studies, FGID symptoms improved during the follow-up period.[51,52] This may be related to treatment in any form (medication or education, etc) or reassurance when visiting a hospital for FGID symptoms. However, as has already been found, FGID symptoms are often associated with other factors, especially psychological factors such as anxiety and depression,[53,54] so that symptoms may not improve or rather worsen during the follow-up period. In a Belgian study in which FD patients were followed- up for 5 years, symptoms improved or disappeared in about half of FD patients, and FD symptoms were associated with anxiety.[52] In an Australian study that followed FGID patients for 12 years, FGID symptoms periodically appeared and disappeared and were closely related to anxiety.[17] Aro et al[16] performed a 10-year follow-up of Swedish FGID patients and reported that patients with anxiety at baseline, but not depression, had a 7.6 fold higher risk of developing FD after 10 years. In the present study, the relationship between anxiety and FGID was confirmed. In overlap FGID, especially in women, and in the overlap of NERD-FD-IBS, the anxiety score was high. In addition, anxiety was associated with epigastric pain, and FGID patients with anxiety did not show significant symptom improvement during the follow-up period, indicating that patients with anxiety did not respond to general FGID treatment, especially in the non-overlap group.

Genetic studies have suggested that polymorphisms in the serotonin transporter (SERT) gene and the G-protein β3 (*GNB3*) C825T gene are associated with FD or IBS. The *GNB3* 825C>T polymorphism has been widely evaluated in FD patients.[55,56] In IBS patients, the *SLC6A4* 5-HTTLPR polymorphism has been well-studied.[57,58] In the present study, the genetic polymorphisms of *SERT* and *GNB3* were observed in NERD-FD-IBS overlap patients and that of the adrenergic receptor gene in NERD-FD patients. The *SERT* gene plays a role in serotonin reuptake by the presynaptic terminal. In the presence of genetic polymorphism, serotonin concentration may rise or fall, thus affecting FGID symptoms. The *SERT* gene was particularly associated with upper GI symptoms such as early satiation and epigastric pain. Among the *SLC6A4* 5-HTTLPR gene polymorphism patients, these symptoms were the most severe in the L/S genotype. In particular, the symptoms of epigastric pain in patients with the *SLC6A4* 5-HTTLPR L/L genotype did not improve during the follow-up period, suggesting that some symptoms affected by genetic polymorphism may not improve even with appropriate treatment. However, chang-

es in FGID clinical symptoms during the follow-up period cannot be explained by genetic polymorphism alone. FGID mechanisms are diverse, and genetic polymorphism comprises only a small part. The patients used various drugs during the follow-up period, and various lifestyle modifications were performed in parallel. However, in some patients, symptoms improved, while in some patients, symptoms worsened. In some patients who did not respond to treatment, polymorphism such as the SERT gene may be related with this prognosis, especially in patients with overlap FGID. However, it is not easy to determine the role of genetic polymorphism by excluding a wide variety of influencing factors. Additional research is needed in the future to clarify this issue.

Our study has several limitations, such as the number of enrolled patients being relatively small especially for the genetic polymorphism study. However, among many neurotransmission-related and cytokine genes, we found that in the presence of the *SLC6A4* 5-HTTLPR L/L genotype alone, the symptoms did not improve during the mean follow-up period of 75.8 months. In addition, *SLC6A4 5-HTTLPR* was found to be associated with female NERD-FD-IBS ($P = 0.024$) patients. This implies that even if this cohort was small, *SLC6A4* 5-HTTLPR L/L seemed to contribute to female patients with overlap FGIDs. The second limitation concerns the definition of NERD. Theoretically, NERD is diagnosed if the 24-hour esophageal pH monitoring is positive without esophageal damage. However, most enrolled patients did not undertake this examination mainly because they refused a 24-hour esophageal pH monitoring. Therefore, clinically, it was difficult to completely differentiate between NERD and RH. This implies that the patients with reflux symptoms were a heterogeneous group.[22]

Actually, RH describe the cases between NERD and functional heartburn.[23,24] However, if the patient visited with GERD symptoms and upper endoscopy ruled out ERD, usually the patient is categorized as NERD simply because we do not know if this patient is NERD or RH or FH without pH-metry or impedance study. Thus we followed this concept in the present study.

Third, there were many patients with follow-up loss. For practical reasons, we received the questionnaire in the endoscopy room whenever patients underwent endoscopy. In Korea, people aged > 40 years are recommended to undergo biannual endoscopy at the National Cancer Control Policy, free of charge. Our hospital is a tertiary institute where endoscopy is not free of charge; thus, if the patients' symptoms disappear, they are less likely to visit the hospital for endoscopy. However, following these examinations, patients with organic diseases were excluded from this study, and only FGID patients were enrolled. Despite these limitations, our study is comprehensive regarding FGIDs related to genetic polymorphisms and questionnaires over long-term follow-up. Fourth, we could not analyze the effects of drugs such as PPI, prokinetics, and anti-spasmotics, which are basically used for FGID, and various neuromodulators that can affect not only FGID symptoms, but also anxiety and depression.

Lastly, since there is no questionnaire to evaluate esophageal symptoms in K-BDQ, the gastroesophageal reflux questionnaire was used to and evaluate the follow-up symptoms of GERD. However, since it is a questionnaire used in some studies and is not officially validated, it is thought that there are some limitations in drawing conclusions from the derived results. However, since it is a questionnaire used in the previous nationwide multi-center study,[25] there may not be a significant difference in the interpretation of the results. In conclusion, patients with overlap FGIDs should be a focus of attention because they are associated with anxiety/depression and more severe symptoms, especially women. Genetic polymorphisms could also be associated with certain symptoms of overlap FGIDs. In overlap FGIDs with certain genetic factors, it may suggest reasons why some symptoms are refractory to lifestyle changes or pharmacological treatment.

Financial support: This research was supported by Support Program for Women in Science, Engineering and Technology through the National Research Foundation of Korea (NRF) funded by the Ministry of Science and ICT (No. 2019H1C3A1032224).

Conflicts of interest: None.

Author contributions: Ju Yup Lee analyzed data and prepared the manuscript drafted; Nayoung Kim designed the study, collected data, and supervised the writing of this manuscript; Ji Hyun Park and Jeong Eun Yu performed genotyping of genetic polymorphisms; Yun Jeong Song and Jung Won Yoon managed and collected the questionnaire data; and Dong Ho Lee supervised the writing of the manuscript. All authors have read and approved the final draft of this article.

References

1. Drossman DA. Functional gastrointestinal disorders: history, pathophysiology, clinical features and Rome IV. Gastroenterology 2016;150:1262-1279.
2. Suzuki H, Hibi T. Overlap syndrome of functional dyspepsia and irritable bowel syndrome - are both diseases mutually exclusive? J Neurogastroenterol Motil 2011;17:360-365.

3. Locke GR 3rd, Zinsmeister AR, Fett SL, Melton LJ 3rd, Talley NJ. Overlap of gastrointestinal symptom complexes in a US community. Neurogastroenterol Motil 2005;17:29-34.
4. Choung RS, Chang JY, Locke GR, Schleck CD, Zinsmeister AR, Talley NJ. Is having multiple functional gastrointestinal disorders distinct from having a single FGID? A population based study. Gastroenterology 2011;140:S708.
5. Ford AC, Marwaha A, Lim A, Moayyedi P. Systematic review and meta-analysis of the prevalence of irritable bowel syndrome in individuals with dyspepsia. Clin Gastroenterol Hepatol 2010;8:401-409.
6. Choung RS, Locke GR, Schleck CD, Zinsmeister AR, Talley NJ. Do distinct dyspepsia subgroups exist in the community? A population-based study. Am J Gastroenterol 2007;102:1983-1989.
7. Agréus L, Svärdsudd K, Nyrén O, Tibblin G. Irritable bowel syndrome and dyspepsia in the general population: overlap and lack of stability over time. Gastroenterology 1995;109:671-680.
8. Perri F, Clemente R, Festa V, et al. Patterns of symptoms in functional dyspepsia: role of Helicobacter pylori infection and delayed gastric emptying. Am J Gastroenterol 1998;93:2082-2088.
9. Stanghellini V, Tosetti C, Paternicò A, et al. Predominant symptoms identify different subgroups in functional dyspepsia. Am J Gastroenterol 1999;94:2080-2085.
10. Talley NJ, Dennis EH, Schettler-Duncan VA, Lacy BE, Olden KW, Crowell MD. Overlapping upper and lower gastrointestinal symptoms in irritable bowel syndrome patients with constipation or diarrhea. Am J Gastroenterol 2003;98:2454-2459.
11. Lee SY, Ryu HS, Choi SC, Jang SH. Psychological factors influence the overlap syndrome in functional gastrointestinal disorder and quality of life among psychiatric patients in South Korea. Psychiatry Investig 2020;17:262-267.
12. Yao X, Yang Y, Zhang S, Shi Y, Zhang Q, Wang Y. The impact of overlapping functional dyspepsia, belching disorders and functional heartburn on anxiety, depression and quality of life of Chinese patients with irritable bowel syndrome. BMC Gastroenterol 2020;20:209.
13. von Wulffen M, Talley NJ, Hammer J, et al. Overlap of irritable bowel syndrome and functional dyspepsia in the clinical setting: prevalence and risk factors. Dig Dis Sci 2019;64:480-486.
14. Aziz I, Palsson OS, Törnblom H, Sperber AD, Whitehead WE, Simrén M. The Prevalence and impact of overlapping Rome IV-diagnosed functional gastrointestinal disorders on somatization, quality of life, and healthcare utilization: a cross-sectional general population study in three countries. Am J Gastroenterol 2018;113:86-96.
15. Berens S, Engel F, Gauss A, et al. Patients with multiple functional gastrointestinal disorders (FGIDs) show increased illness severity: a cross-sectional study in a tertiary care FGID specialty clinic. Gastroenterol Res Pract 2020;2020:9086340.
16. Aro P, Talley NJ, Johansson SE, Agréus L, Ronkainen J. Anxiety is linked to new-onset dyspepsia in the Swedish population: a 10-year follow-up study. Gastroenterology 2015;148:928-937.
17. Koloski NA, Jones M, Kalantar J, Weltman M, Zaguirre J, Talley NJ. The brain--gut pathway in functional gastrointestinal disorders is bidirectional: a 12-year prospective population-based study. Gut 2012;61:1284-1290.
18. Adam B, Liebregts T, Holtmann G. Mechanisms of disease: genetics of functional gastrointestinal disorders--searching the genes that matter. Nat Clin Pract Gastroenterol Hepatol 2007;4:102-110.
19. Choi YJ, Hwang SW, Kim N, Park JH, Oh JC, Lee DH. Association between SLC6A4 serotonin transporter gene lainked polymorphic region and ADRA2A -1291C>G and irritable bowel syndrome in Korea. J Neurogastroenterol Motil 2014;20:388-399.
20. Hwang SW, Kim N, Jung HK, et al. Association of SLC6A4 5-HTTLPR and TRPV1 945G>C with functional dyspepsia in Korea. J Gastroenterol Hepatol 2014;29:1770-1777.
21. Choi YJ, Kim N, Yoon H, et al. Overlap between irritable bowel syndrome and functional dyspepsia including subtype analyses. J Gastroenterol Hepatol 2017;32:1553-1561.
22. Savarino E, Zentilin P, Savarino V. NERD: an umbrella term including heterogeneous subpopulations. Nat Rev Gastroenterol Hepatol 2013;10:371-380.
23. Hungin APS, Molloy-Bland M, Scarpignato C. Revisiting Montreal: new insights into symptoms and their causes, and implications for the future of GERD. Am J Gastroenterol 2019;114:414-421.
24. Aziz Q, Fass R, Gyawali CP, Miwa H, Pandolfino JE, Zerbib F. Functional esophageal disorders. Gastroenterology 2016;150:1368-1379.
25. Lee JH, Kim N, Chung IK, et al. Clinical significance of minimal change lesions of the esophagus in a healthy Korean population: a nationwide multi-center prospective study. J Gastroenterol Hepatol 2008;23:1153-1157.
26. Kim N, Lee SW, Cho SI, et al. The prevalence of and risk factors for erosive oesophagitis and non-erosive reflux disease: a nationwide multicentre prospective study in Korea. Aliment Pharmacol Ther 2008;27:173-185.
27. Park JJ, Kim JW, Kim HJ, et al. The prevalence of and risk factors for Barrett's esophagus in a Korean population: a nationwide multicenter prospective study. J Clin Gastroenterol 2009;43:907-914.
28. Shim KN, Hong SJ, Sung JK, et al. Clinical spectrum of reflux esophagitis among 25,536 Koreans who underwent a health check-up: a nationwide multicenter prospective, endoscopy-based study. J Clin Gastroenterol 2009;43:632-638.
29. Lee ES, Kim N, Lee SH, et al. Comparison of risk factors and clinical responses to proton pump inhibitors in patients with erosive oesophagitis and non-erosive reflux disease. Aliment Pharmacol Ther 2009;30:154-164.
30. Fass R, Fennerty MB, Vakil N. Nonerosive reflux disease--current concepts and dilemmas. Am J Gastroenterol 2001;96:303-314.
31. Noh YW, Jung HK, Kim SE, Jung SA. Overlap of erosive and nonerosive reflux dseases with functional gastrointestinal disorders according to Rome III criteria. J Neurogastroenterol Motil 2010;16:148-156.
32. Tack J, Talley NJ, Camilleri M, et al. Functional gastroduodenal disorders. Gastroenterology 2006;130:1466-1479.
33. Vakil N, Halling K, Ohlsson L, Wernersson B. Symptom overlap between postprandial distress and epigastric pain syndromes of the Rome III dyspepsia classification. Am J Gastroenterol 2013;108:767-774.
34. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterology

2006;130:1480-1491.
35. Song KH, Jung HK, Min BH, et al. Development and validation of the Korean Rome III questionnaire for diagnosis of functional gastrointestinal disorders. J Neurogastroenterol Motil 2013;19:509-515.
36. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol 1997;32:920-924.
37. Kim JY, Kim N, Seo PJ, et al. Association of sleep dysfunction and emotional status with gastroesophageal reflux disease in Korea. J Neurogastroenterol Motil 2013;19:344-354.
38. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res 2002;52:69-77.
39. Lee SY, Lee KJ, Kim SJ, Cho SW. Prevalence and risk factors for overlaps between gastroesophageal reflux disease, dyspepsia, and irritable bowel syndrome: a population-based study. Digestion 2009;79:196-201.
40. Park H. Functional gastrointestinal disorders and overlap syndrome in Korea. J Gastroenterol Hepatol 2011;26(suppl 3):12-14.
41. Corsetti M, Caenepeel P, Fischler B, Janssens J, Tack J. Impact of coexisting irritable bowel syndrome on symptoms and pathophysiological mechanisms in functional dyspepsia. Am J Gastroenterol 2004;99:1152-1159.
42. Wang A, Liao X, Xiong L, et al. The clinical overlap between functional dyspepsia and irritable bowel syndrome based on Rome III criteria. BMC Gastroenterol 2008;8:43.
43. Lee HJ, Lee SY, Kim JH, et al. Depressive mood and quality of life in functional gastrointestinal disorders: differences between functional dyspepsia, irritable bowel syndrome and overlap syndrome. Gen Hosp Psychiatry 2010;32:499-502.
44. Bennett EJ, Piesse C, Palmer K, Badcock CA, Tennant CC, Kellow JE. Functional gastrointestinal disorders: psychological, social, and somatic features. Gut 1998;42:414-420.
45. Lee SP, Sung IK, Kim JH, Lee SY, Park HS, Shim CS. The effect of emotional stress and depression on the prevalence of digestive diseases. J Neurogastroenterol Motil 2015;21:273-282.
46. Thijssen AY, Jonkers DM, Leue C, et al. Dysfunctional cognitions, anxiety and depression in irritable bowel syndrome. J Clin Gastroenterol 2010;44:e236-e241.
47. Pinto-Sanchez MI, Ford AC, Avila CA, et al. Anxiety and depression increase in a stepwise manner in parallel with multiple FGIDs and symptom severity and frequency. Am J Gastroenterol 2015;110:1038-1048.
48. Foster JA, McVey Neufeld KA. Gut-brain axis: how the microbiome influences anxiety and depression. Trends Neurosci 2013;36:305-312.
49. Vanuytsel T, van Wanrooy S, Vanheel H, et al. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell-dependent mechanism. Gut 2014;63:1293-1299.
50. Larauche M. Novel insights in the role of peripheral corticotropin-releasing factor and mast cells in stress-induced visceral hypersensitivity. Neurogastroenterol Motil 2012;24:201-205.
51. Ivashkin VT, Poluektova EA, Glazunov AB, Putilovskiy MA, Epstein OI. Pathogenetic approach to the treatment of functional disorders of the gastrointestinal tract and their intersection: results of the Russian observation retrospective program COMFORT. BMC Gastroenterol 2019;20:2.
52. Kindt S, Van Oudenhove L, Mispelon L, Caenepeel P, Arts J, Tack J. Longitudinal and cross-sectional factors associated with long-term clinical course in functional dyspepsia: a 5-year follow-up study. Am J Gastroenterol 2011;106:340-348.
53. Koloski NA, Talley NJ, Boyce PM. Does psychological distress modulate functional gastrointestinal symptoms and health care seeking? A prospective, community Cohort study. Am J Gastroenterol 2003;98:789-797.
54. Alander T, Svärdsudd K, Johansson SE, Agréus L. Psychological illness is commonly associated with functional gastrointestinal disorders and is important to consider during patient consultation: a population-based study. BMC Med 2005;3:8.
55. Saito YA, Mitra N, Mayer EA. Genetic approaches to functional gastrointestinal disorders. Gastroenterology 2010;138:1276-1285.
56. Oshima T, Toyoshima F, Nakajima S, Fukui H, Watari J, Miwa H. Genetic factors for functional dyspepsia. J Gastroenterol Hepatol 2011;26(suppl 3):83-87.
57. Saito YA. The role of genetics in IBS. Gastroenterol Clin North Am 2011;40:45-67.
58. Camilleri M, Katzka DA. Irritable bowel syndrome: methods, mechanisms, and pathophysiology. Genetic epidemiology and pharmacogenetics in irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2012;302:G1075-G1084.