ORIGINAL ARTICLE

Gut and Liver, Vol. 6, No. 2, April 2012, pp. 223-228

Polymorphisms of the Serotonin Transporter Gene and G-Protein β3 Subunit Gene in Korean Children with Irritable Bowel Syndrome and Functional Dyspepsia

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Background/Aims: Many candidate gene studies have revealed that polymorphisms of the 5'-flanking controlled SERT gene linked polymorphic region (5HTT-LPR) gene and G-protein β3 C825T gene might be associated with functional dyspepsia (FD) and irritable bowel syndrome (IBS). This study was performed to investigate polymorphisms of the 5HTT-LPR gene and G-protein β3 C825T gene in FD and IBS in Korean children. **Methods:** In total, 102 patients with FD, 72 patients with IBS based on the Rome III criteria and 148 healthy controls without gastrointestinal symptoms were included in the study to analyze 5HTT-LPR and G-protein β3 C825T polymorphisms. **Results:** 5HTT-LPR genotype analysis revealed no significant differences in FD and IBS patients compared with controls. The GNβ3 C825T genotype distribution for CC, CT, and TT was 23.6%, 53.4%, and 23.0% in controls, 36.3%, 38.2%, and 25.5% in FD and 37.5%, 38.9%, and 23.6% in IBS, respectively. The CC genotype was more common in FD and IBS patients than controls ($p<0.05$). When the IBS patients were grouped according to IBS subtypes, CC genotype GNβ3 C825T was common in diarrhea-dominant IBS, and the TT genotype was common in constipation-dominant IBS ($p<0.05$). **Conclusions:** The CC genotype of G-protein β3 C825T may be associated with FD and diarrhea-predominant IBS. The TT genotype may be associated with constipation-predominant IBS. **(Gut Liver 2012;6:223-228)**

Key Words: Functional dyspepsia; Irritable bowel syndrome; Serotonin transporter; G-protein; Genotype

INTRODUCTION

Functional gastrointestinal disorder (FGID) is characterized by structural and biochemical dysfunctions and has various and repetitive gastrointestinal symptoms without definite pathophysiology. Therefore, it can only be diagnosed by characteristic symptoms or after excluding other diseases with laboratory results or procedures such as endoscopy.[1] Functional dyspepsia (FD) and irritable bowel syndrome (IBS) are well known pathologies of FGID; both are highly prevalent in up to 25% of the population. Many studies of patients with FD and IBS have shown functional disturbances in gastrointestinal motor and sensory function. The etiology of FGID is assumed to be associated with infection, alterations of the immune system or intestinal motility, or even psychiatric factors.[2,3] Recently, genetic approaches have made forward steps to identify the etiology. For example, many family aggregation and twin studies have reported genetic components that might be associated with FGID, although common environmental factors they might also share must be considered.[4,5]

A number of receptors have been proven to have altered functions in FGID, including cholecystokinin, serotonin transporter (SERT) protein, heterotrimeric G-proteins, and interleukin (IL)-10. Among many candidate genes, the SERT protein gene is the best known in IBS, and there is a part called the 5'-flanking controlled SERT gene linked polymorphic region (5HTT-LPR), which reveals the SERT protein.[6] Serotonin (5-HT) plays a key role in modulating sensory and motor functions in the gastrointestinal tract. In particular, 5-HT type 3 receptors are known to mediate the postprandial colonic motor responses, cramping, diarrhea, and constipation in IBS patients. A prior study in Korea demonstrated SS subtype of SERT is significantly associated with diarrhea-predominant IBS in adults.[7]

G-protein is another emerging candidate gene to study and is essential for stimulus-response coupling in the intracellular system; it is involved in ion channels and protein kinases. It is

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Received on July 6, 2011. Revised on September 7, 2011. Accepted on October 18, 2011.
pISSN 1976-2283 eISSN 2005-1212 http://dx.doi.org/10.5009/gnl.2012.6.2.223

also a main mediator in controlling a signal transport into the cellular system. G-Protein β3 C825T polymorphism could lead to an altered signal transduction response and functional abnormality such as changes of sensory function or motility associated with FGID.[8] GNβ3 has a single nucleotide polymorphism in C825T, which converts cytosine to thymidine, and allele shifting was performed according to each subtype, each of which has a different activity. With even a small change, GNβ3 C825T CC type plays a role in the reduction of signaling, subsequently alters gastrointestinal sensation and motility, and is therefore considered to be associated with FD or IBS.[9] TT type potentiates G-protein activity and cellular reactions that can cause cardiovascular disease, hypertension, metabolic disease, and affective disorder.[10]

In this study, we tried to investigate that the polymorphisms of SERT and GNβ3 C825T would be associated with IBS and FD in Korean children.

MATERIALS AND METHODS

1. Study subjects

Patients aged 4 to 18 years who visited outpatient clinic of the Department of Pediatrics at Eulji Medical Center were recruited consecutively from November 2009 to July 2010. All patients underwent validated questionnaires regarding gastrointestinal symptoms according to Rome III criteria. The study subjects were diagnosed as IBS or FD by their responses to questionnaire.[11] In children and adolescents, FD is defined as persistent or recurrent pain or discomfort centered in the upper abdomen (above the umbilicus) not relieved by defecation or associated with the onset of a change in stool frequency or stool form. IBS is defined as abdominal discomfort or pain associated with 2 or more of the followings at least 25% of the time; improved with defecation, onset associated with a change in frequency of stool, onset associated with a change in form of stool. Both criteria are fulfilled when the symptom occurs at least once per week for at least 2 months before diagnosis.[11]

For each participant, detailed history taking, physical examination, and if necessary, further evaluation such as blood tests, abdominal ultrasonography, and upper gastrointestinal endoscopy were performed to distinguish FGIDs from other organic causes. Exclusion criteria included significant upper gastrointestinal diseases such as gastrointestinal bleeding, persistent vomiting, peptic ulcer disease and reflux esophagitis. Severe systemic diseases that may induce gastrointestinal symptoms, lactase deficiency, a history of previous major abdominal surgery and developmental disability were also excluded. Healthy children who visited the clinic for screening purposes without gastrointestinal symptoms were invited to participate as a control group.

Finally, 174 FGID patients (72 with IBS and 102 with FD) and 148 control subjects participated in this study.

All patients were provided with written informed consent for the study. This prospective study was approved by the Institutional Review Board of Eulji University School of Medicine, which confirmed that the study was in accordance with the ethical guidelines of the Helsinki Declaration.

2. Methods

1) Genomic DNA preparation

Genomic DNA was prepared from peripheral blood samples using a nucleic acid isolation device, QuickGene-mini80 (Fujifilm, Tokyo, Japan).

2) Genotyping for GNβ3 C825T

The genotyping was screened using single base primer extension assay using ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to manufacturer's recommendation. The DNA fragment amplification was conducted with the sense primer 5'-TGGCACGTGGTATGTGTTG-3' and the antisense primer 5'-GGAACCAAGGGGTACTGGA-3'.

Briefly, the genomic DNA flanking the interested single-nucleotide polymorphism was amplified with polymerase chain reaction (PCR) with Forward and Reverse primer pairs and standard PCR reagents in 10 μL reaction volume, containing 10 ng of genomic DNA, 0.5 pM of each oligonucleotide primer, 1 μL 10x PCR buffer, 250 μM dNTP (2.5 mM each) and 0.25 unit i-StarTaq DNA Polymerase (5 unit/μL) (iNtRON Biotechnology, Seongnam, Korea). The PCR reactions were carried out as follows: 10 minutes at 95°C for 1 cycle, and 35 cycles on 95°C for 30 seconds, 60°C for 1 minute, 72°C for 1 minute followed by 1 cycle of 72°C for 10 minutes. After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP) (USB Co., Cleveland, OH, USA) and exonuclease I (USB Co.) at 37°C for 75 minutes and 72°C for 15 minutes to purify the amplified products. One microliter of the purified amplification products were added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pmols of genotyping primer for primer extension reaction. The primer extension reaction was carried out for 25 cycles of 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 30 seconds. The reaction products were treated with 1 unit of SAP at 37°C for 1 hour and at 72°C for 15 minutes to remove excess fluorescent dye terminators. One microliter of the final reaction samples containing the extension products were added to 9 μL of Hi-Di formamide (ABI). The mixture was incubated at 95°C for 5 minutes, followed by 5 minutes on ice and then analyzed by electrophoresis in ABI Prism 3730xl DNA analyzer. Analysis was carried out using Genemapper software (version 4.0; Applied Biosystems, Foster City, CA, USA).

3) Genotyping for SERT

PCR was carried out in a total volume of 10 μL containing 10 ng genomic DNA, 0.5 uM each of the sense (5'-GGCGTTGCCGCTCTGAATGC-3') and antisense (5'-GAGGGACTGAGCTGGACAACCAC-3') primers, 0.5 mM each of four deoxynucleotide

phosphates (dATP, dCTP, dGTP, dTTP), 0.25 unit i-StarTaq DNA Polymerase. To amplify the fragment, the 500 mM of betaine was added to the PCR system. Amplification conditions consisted of an initial denaturing step at 95°C followed by 35 cycles of 95°C for 30 seconds, 65°C for 1 minute and 72°C for 1 minute. This was followed by a final extension step at 72°C for 10 minutes. To detect the amplified DNA fragment, we analyzed 2 μL of the reaction mixtures on a 3% agarose gel (ReadyAgarose 96 plus 3% TBE Gel; Bio-Rad, Richmond, CA, USA).

4) Statistical analysis

We calculated allele and genotype frequencies (%) for each polymorphism among controls and each group of patients. Differences of these frequencies were compared using chi-square test or Student's t-test. A logistic regression analysis was performed to test the association between genotype distribution of polymorphism for SERT and Gnβ3 C825T and diseases of FD and IBS. The odds ratio (OR) and 95% confidence intervals (CI) were calculated by logistic regression. SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Statistical significance was determined as p-value <0.05.

RESULTS

Characteristics of study subjects are shown in Table 1. Seventy-two IBS patients and 102 FD patients who met the Rome III criteria were enrolled. There was no statistical difference with respect to age and gender among IBS, FD and control group (Table 1). Of the 72 IBS cases, 61% had diarrhea-predominant IBS, 24% had constipation-predominant IBS and 15% had alternating- type IBS.

1. IBS vs controls genotype distribution

The 5HTT-LPR genotype distribution for LL, LS, and SS was 1.4%, 37.5%, and 61.1%. SS type was more common in IBS group than in controls, but the difference was not significant.

The genotype frequencies for GNβ3 C825T polymorphism was 37.5%, 38.9% and 23.6% for CC, CT, and TT. CC genotype was more common than in controls (p<0.05) (OR, 1.937; 95% CI, 1.053 to 3.563) (Table 2).

Table 1. Demographic Data of the Study Population

	Controls (n=148)	IBS (n=72)	FD (n=102)
Gender			
Male	81 (54.7)	32 (44.4)	32 (31.3)
Female	67 (45.3)	40 (55.6)	70 (68.7)
Male:Female*	1.2:1	0.8:1	0.5:1
Age, mean±SD, yr*	10.8±3.9	13.3±3.7	11.2±3.6

Data are presented as number (%).
IBS, irritable bowel syndrome; FD, functional dyspepsia; SD, standard deviation.
*p>0.05.

2. FD vs controls genotype distribution

The 5HTT-LPR genotype distribution for LL, LS, and SS was 3.4%, 37.2%, 59.5% in controls and 8.8%, 27.5%, 63.7% in FD. LL and SS types were more common in FD group than in controls, but the difference was not significant.

The GNβ3 C825T genotype distribution for CC, CT, and TT was 23.6%, 53.4%, 23.0% in controls and 36.3%, 38.2%, 25.5% in FD. CC genotype was more common than in controls (p<0.05) (OR, 1.838; 95% CI, 1.056 to 3.197) (Table 2).

3. IBS-subtype genotype distribution

Genotype frequency differences were not observed for 5HTT-LPR polymorphism between the IBS subtypes and controls. CC genotype of GNβ3 C825T was common in diarrhea predominant IBS (p<0.05) (OR, 2.69; 95% CI, 1.330 to 5.441) and TT genotype was common in constipation predominant IBS (p<0.05) (OR, 2.98; 95% CI, 1.068 to 8.319) (Table 3).

DISCUSSION

Several polymorphisms for genes have been studied to identify the etiology of functional gastrointestinal diseases, and there are several candidate genes such as SERT gene, G-protein, IL-10, tumor necrosis factor (TNF)-α, and adrenalin that play

Table 2. Polymorphisms of SERT and the GNβ3 C825T Gene among the Control, IBS and FD Patients

Genotype	Controls (n=148)	IBS (n=72)	FD (n=102)
5HTT-LPR polymorphism, n (%)			
LL	5 (3.4)	1 (1.4)	9 (8.8)
LS	55 (37.2)	27 (37.5)	28 (27.5)
SS	88 (59.5)	44 (61.1)	65 (63.7)
L-allele	65	29	46
S-allele	231	115	158
GNβ3 C825T polymorphism, n (%)			
CC	35 (23.6)	27 (37.5)*	37 (36.3)†
CT	79 (53.4)	28 (38.9)	39 (38.2)
TT	34 (23.0)	17 (23.6)	26 (25.5)
C-allele	149	82	113
T-allele	147	62	91

SERT, serotonin transporter; IBS, irritable bowel syndrome; FD, functional dyspepsia; 5HTT-LPR, 5'-flanking controlled SERT gene linked polymorphic region.
*χ^2=7.639, p=0.022 (FD vs controls), odds ratio (95% confidence interval) for CC vs non-CC genotypes: 1.838 (1.056-3.197); †χ^2=6.435, p=0.040 (IBS vs controls), odds ratio (95% confidence interval) for CC vs non-CC genotypes: 1.937 (1.053-3.563).

Table 3. Polymorphisms of SERT and GNβ3 C825T among the Controls and Each of the IBS Subtypes

Genotype	Controls (n=148)	D-IBS (n=44)	C-IBS (n=17)	A-IBS (n=11)
5HTT-LPR polymorphism, n (%)				
LL	5 (3.4)	0 (0.0)	0 (0.0)	1 (9.1)
LS	55 (37.2)	19 (43.2)	7 (41.2)	4 (36.4)
SS	88 (59.5)	25 (56.8)	10 (58.8)	6 (54.5)
L-allele	65	19	7	6
S-allele	231	69	27	16
GNβ3 C825T polymorphism, n (%)				
CC	35 (23.6)	20 (45.5)*	2 (11.8)	5 (45.4)
CT	79 (53.4)	17 (38.6)	7 (41.2)	4 (36.4)
TT	34 (23.0)	7 (15.9)	8 (47.1)†	2 (18.2)
C-allele	149	57	11	14
T-allele	147	31	23	10

SERT, serotonin transporter reuptake gene; IBS, irritable bowel syndrome; D-, diarrhea dominant; C-, constipation dominant; A-, alternating constipation and diarrhea; 5HTT-LPR, 5'-flanking controlled SERT gene linked polymorphic region.
*χ^2=8.280, p=0.016 (control vs D-IBS), odds ratio (95% confidence interval) for CC vs non-CC genotypes: 2.69 (1.330-5.441); †χ^2 =7.633, p=0.006 (control vs C-IBS), odds ratio (95% confidence interval) for TT vs non-TT genotypes: 2.98 (1.068-8.319).

important roles in the pathophysiology of FGID.[12] This study aimed to investigate that polymorphism of SERT and G-protein was differently expressed in children with FGID. SERT gene is most frequently studied gene in IBS, and three different polymorphic sites have been found. The function of SERT in the gastrointestinal tract is similar to its function in the brain. SERT polymorphism occurs as a 44-base-pair insertion or deletion of the SERT gene. 5HTT-LPR of the 5'flanking controlling site, variable number tandem repeats in the second intron, and rs25531 have thus far been studied. In particular, the S subtype of 5HTT-LPR is known to have longer serotonin action time compared to the L subtype because of reduced transcription ability against SERT.[13] Yeo *et al*.[14] reported that the SS type was associated with diarrhea-predominant IBS, but other studies conducted in Western countries showed that there was no relationship between IBS and 5HTT-LPR.[15,16] Our study showed lack of association between 5HTT-LPR genotypes and all types of IBS. The discrepancies may be due to racial or regional differences and we can assume that although these polymorphisms may have clinical consequences, their effects are not significant. Previous studies with FD patients showed that there was no close relationship between SERT and FD.[12,17] Our data was consistent with previous data and there were no definite differences between this polymorphism and FD.

G-proteins are known as key receptors that comprise about 80% of cellular receptors and play an important role in transporting signals into the cell. Signal transporting is regulated through changes of G-protein.[18] Holtmann *et al*.[19] studied 67 patients with epigastric pain of unknown origin and reported that homozygous GNβ3 C825T CC genotype was associated more strongly with FD than controls. Camilleri *et al*.[12] reported that homozygous CC or TT genotypes are associated with meal unrelated dyspepsia due to fluctuations of G-protein activity. But it is unlikely that subjects presenting with same dyspepsia phenotype group reveal the association with both alleles. Other studies have reported that the TT type was related to FD.[17,20] These contrasting findings may be due to differences of genotype composition in different countries, which comprise different racial groups. In our study, the CC genotype was more prevalent in FD group than the control group and considered statistically significant (p<0.05). Our result of FD patients was coincided with that of Holtmann *et al*.[9] CC genotype might inhibit the translation of G-protein and also the activity against neurotransmitters such as serotonin at 5HT4 receptors which stimulates motor function and may cause gastric motility disorder such as gastroparesis.

Previous studies in Western countries have reported that there was no correlation between GNβ3 C825T and IBS.[21,22] But recently a study with Korean adults showed that GNB3 825T allele might be associated with IBS with constipation and in a Greek study TT genotype and T allele of GNβ3 showed significant association with IBS.[23,24]

We observed CC type was more common in overall IBS than in controls. When the IBS patients were divided into subgroups of diarrhea dominant IBS, constipation dominant IBS and alternating type IBS, CC genotype was associated with diarrhea dominant IBS and TT genotype was associated with constipation dominant IBS. GNβ3 T allele can be associated with visceral hypersensitivity as a consequence of the increased signal transduction upon G-protein-coupled receptors activation and may be related with symptom generation in IBS.[25] Alternately, CC genotype is known to result in reduced G protein translation. Besides subjects with CC genotype may have decreased immune response to infection and thus needs prolonged period of recovery from any infection. This may act as predisposing factor to

visceral hypersensitivity.[9]

Our data are not consistent with previous studies that GNβ3 CC genotype was not associated with IBS. The different result between previous studies and our study are might be due to racial difference or small number of patients tested.

There are some limitations to this study. The sample size was not large enough to identify possible genetic associations. However, to our knowledge, this is the first study to demonstrate genetic polymorphisms in children with FGID. If there are genetic factors contributing to the manifestation of FGID, investigation in children will be more important than in adults. If we can assume that people develop FGID before they show clinical symptoms, genetic testing may facilitate diagnosis to save economic costs for invasive procedures to discover the cause of gastrointestinal symptoms from the onset.

FGIDs are common in children, accounting for 5% to 10% of pediatric primary practice consultation and 25% of pediatric gastroenterology consultation.[26] Despite the high prevalence and effects of FGIDs, no evidence-based guidelines for its evaluation and treatment exist. If FGIDs are associated with genetic factors, and if some children have predispositions of FGIDs, the facts would affect their adult's life. In this point, the follow-up study would be important. The relation of treatment response or prognosis of FGIDs with the subtypes of polymorphism is also necessary.

In conclusion, the present study suggests that there is significant correlation between polymorphisms of the G-protein β3 C825T genotypes and FD. FD might result partly from genetically altered G-protein receptor coupling. Additional environmental or genetic background might be involved. The association of SERT genotype with FD or IBS phenotype compared with controls was not statistically significant. For the future, there needs to be more data from more diverse areas and a larger sample to verify and analyze the possible involvement of candidate genes that influence the development of FGIDs.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGEMENTS

This study was supported by 2009 Eulji Research Grant (EJRG-09-015-11E13).

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