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. 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. 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.

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. 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.

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
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. 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. 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 intestinal symptoms. The study patients underwent validated questionnaires regarding gastrointestinal symptoms in Korean children.

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 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’-TGGCACGTGTATGTGTTG-3’ and the antisense primer 5’-GGAACCAAGGGTGACTGGA-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 μM of each oligonucleotide primer, 1 μL of PCR buffer, 250 μM dNTP (2.5 mM each) and 0.25 unit i-StarTaq DNA Polymerase (5 units/μL) (IniRON 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 μM each of the sense (5’-GGCGTTGCG-GCTCTGAAATGC-3’) and antisense (5’-GAGGGACTGAGCTGGAGA-3’) primers, 0.5 mM each of four deoxynucleotide