**Name of journal:** ***World Journal of Gastroenterology***

**Manuscript NO: 36608**

**Manuscript Type: ORIGINAL ARTICLE**

***Basic Study***

**Effect of *Lactobacillus rhamnosus* GG supernatant on serotonin transporter expression in rats with post-infectious irritable bowel syndrome**

Cao YN *et al*.LGG-s on SERT for rats with PI-IBS

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**Author contributions:** Cao Yn and Feng Lj contributed essential research and statistical analysis; Wang Ym, Jiang K and Wang Bm designed the study; Zhang Mj, Gu Yx and Liu Yy were mainly responsible for cultivation of bacteria, Gao J, Liu Yy and Wang Zl contributed raising animals; Cao Yn and Feng Lj edited the article; the financial arrangement was supported by Wang Ym.

**supported by** the National Natural Science Foundation of China, No. 81570489.

**Institutional review board statement:** After examination, this study was approved by the Tianjin Medical University General Hospital.

**Institutional animal care and use committee statement:** After inspection, all procedures involving animals were reviewed and approved by the Animal Ethical and Welfare of Tianjin Medical University.

**Conflict-of-interest statement:** The authors declare that they have no competing interests.

**Data sharing statement:** Readers can get the data of this paper by contacting us with E-mail: ywang12@tmu.edu.cn.

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**Manuscript source:** Unsolicited manuscript

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**Received:** November 9, 2017

**Peer-review started:** November 9, 2017

**First decision:** November 30, 2017

**Revised:** December 6, 2017

**Accepted:** December 13, 2017

**Article in press:**

**Published online:**

**Abstract**

***AIM***

To evaluate the affection of *Lactobacillus rhamnosus* GG supernatant (LGG-s) on SERT in the PI-IBS rats.

***Methods***

1010 cfu/ml *Campylobacter jejuni* 81-176 was used to make an intestinal-infection to build the PI-IBS models. After evaluation of the post-infectious phase by biochemical tests, DNA agarose gel electrophoresis, abdominal withdrawal reflex (AWR) test and the intestinal motility test, the control and four PI-IBS groups received supplements of different concentrations of LGG-s for 4 wk. Rats were maintained in treatments for 1.0, 2.0, 3.0, 4.0 wk during the experiment, the colons and brains were removed for later use each week. SERT-mRNA and SERT-P levels were detected by Real-Time-PCR and Western-blotting.

***RESULTS***

The levels of SERT-mRNA and SERT-P were higher than those in control-group and PI-IBS gavaged with PBS-group in intestines of rats during the whole study. Undiluted-LGG-s up-regulated SERT-mRNA levels by 2.67-times the control-group by second week and continued increasing. Double-diluted LGG-s was similar to undiluted-LGG-s, maintaining a high-level of SERT-mRNA. Triple-diluted LGG-s up-regulated SERT-mRNA expression levels by 6.9-times the control-group, but decreased rapidly at the end of second week. SERT-P levels were basically flat for undiluted-LGG-s, double-diluted LGG-s and triple-diluted LGG-s, higher than control-group and PI-IBS PBS group. It was basically flat for B, C and D by the second and third week in the intestines of rats. SERT-mRNA and SERT-P levels in the brain of the rats had no statistical significance in the groups during the experiment.

***CONCLUSION***

LGG-s could up-regulate SERT mRNA and SERT-P levels in rat intestinal tissues and has no influence in rat brain tissues.

**Key words** Irritable bowel syndrome; serotonin-transporter; intestinal infection; *Lactobacillus rhamnosus* supernatant

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**C****ore tip:** There are few reports about the effect of the supernatant of *Lactobacillus rhamnosus* GG (LGG) on serotonin transporter (SERT) in rats with post-infectious irritable bowel syndrome (PI-IBS). Experimental models of PI-IBS rats were built with *C. jejuni* infection. SERT levels in intestinal and brain tissues were detected to evaluate the effect of the LGG-s.

Cao YN, Feng LJ, Liu YY, Jiang K, Zhang MJ, Gu YX, Wang BM, Gao J, Wang ZL, Wang YM. Effect of *Lactobacillus rhamnosus* GG supernatant on serotonin transporter expression in rats with post-infectious irritable bowel syndrome. *World J Gastroenterol* 2017; In press

**INTRODUCTION**

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder (FGD), annually affecting 12% to 30% of the population worldwide[[1-5](#_ENREF_1)]. IBS can be divided into 4 subtypes, namely IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), IBS mixed type (IBS-M) and IBS untyped (IBS-U)[[6-8](#_ENREF_6)]. Acute infectious gastroenteritis (IGE) is an important risk factor to develop IBS, with 5% to 31% of the patients developing post-infectious IBS (PI-IBS)[[9-12](#_ENREF_9)]. Recent reports indicate abnormalities in serotonergic signaling systems being involved in the development of PI-IBS, particularly those affecting serotonin (5-HT) levels in the gastrointestinal tract[[13-15](#_ENREF_13)].

As a signal transducer and a neurotransmitter, 5-HT modulates intestinal fluid secretion, gut motility and gastrointestinal sensation[[16](#_ENREF_16)]. Serotonin transporter (SERT) is a universally existing cross-membrane transport protein that plays a key role in 5-HT reuptake[[15](#_ENREF_15),[17](#_ENREF_17)]. SERT has two important polymorphic areas. First, named 5-HTTLPR,which is in the regulatory region of its gene (SLC6A4; chromosome 17q11.1–q12)[[18](#_ENREF_18)]. The most frequently studied variant is subdivided into long (L) and short (S) alleles[[19](#_ENREF_19),[20](#_ENREF_20)]. The transcriptional efficiency of the L/L/genotype is significantly higher than the L/S and S/S genotypes[[21](#_ENREF_21)]. Furthermore, the frequency of the L/L genotype in C-IBS was significantly higher than that in the D-IBS, and the S/S genotype is higher in D-IBS[[21-24](#_ENREF_21)]. In other words, the expression of SERT is higher in C-IBS, and lower in D-IBS. Second, named STin2, also called variable number tandem repeats (VNTR). Wang *et al*[[25](#_ENREF_25)] has found a higher ratio of STin2.12/10 and a lower ratio of STin2.12/12 in IBS patients, and no significant differences between different subtypes. However, small scale study about SERT in PI-IBS, conducted by Wheatcroft *et al*[[26](#_ENREF_26)], showed that SERT expression reduced in PI-IBS patients.

*Lactobacillus rhamnosus* GG (LGG) is the best studied member of the lactic acid bacteria and is known to have positive effects on human health[[27](#_ENREF_27)]. Probiotics such as LGG are potential treatment options in patients with IBS[[28](#_ENREF_28)]. LGG could exclude pathogens, promote mucosal immunity to Salmonella infection[[29](#_ENREF_29)] and reduce the rotavirus-related diarrhea by increasing the levels of IFN-γ[[30](#_ENREF_30)]. The ESPGHAN Working Group (WG) recommends using LGG for preventing nosocomial diarrhea[[31](#_ENREF_31)]. Furthermore, LGG reduces the frequency and severity of abdominal pain in children with IBS[[32](#_ENREF_32)], along with improving disease severity, especially in IBS-D and IBS-A subtypes[[33](#_ENREF_33)].

Our previous study had confirmed that Lactobacillus rhamnosus GG supernatant (LGG-s) could up-regulate SERT mRNA and protein levels in intestinal epithelial cells and mice intestinal tissues[[34](#_ENREF_34)]. The aim of this study was to investigate the effects of LGG-s on the expression of SERT mRNA and SERT protein (SERT-P) in colon and brain in a rat model of PI-IBS.

**MATERIALS AND METHODS**

***Bacterial culture, LGG-s, Campylobacter jejuni***

LGG (53103, American type culture collection, ATCC, United States) was incubated in Lactobacillus MRS broth (Oxoid CM0359) at 37 ℃ for 24 h, according to ATCC guidelines, diluted in MRS broth, and cultured again at 37 ℃ to reach log phase with the density determined as 0.5 at A600[[34](#_ENREF_34)]. The culture suspension was centrifuged at 4000 *g* for 15 min, then the supernatant was collected and filtered through 0.20-μm filters[[35](#_ENREF_35)].

*Campylobacter jejuni* (*C. jejuni*) 81-176 (BAA-2151, American type culture collection, ATCC, US) was grown on Skirrow's selective medium (Columbia Agar Base, Oxoid CM0331, supplemented with 5% sheep blood and *Campylobacter* selective supplement, Oxoid SR0117) at 42 °C under micro-aerobic conditions for 24 h. The bacterial colony was obtained by an inoculating loop, diluted in phosphate buffered solution (PBS), until the concentration reached 1.0 × 1010 cfu/ml. The preliminary experiments showed that the concentration at 1010cfu/ml got a higher diarrhea rate, visceral hypersensitivity and serious clinical symptoms. Bacterial concentrations were measured by spectrophotometer (TECAN infinite M200 PRO, Switzerland)[[36](#_ENREF_36)].

***Animal studies***

The study was performed on male Sprague-Dawley rats obtained from Laboratory animal center of Chinese People’s Liberation Army General Hospital (Beijing, China), which were aged between 7and 8 wk. The rats were kept under the following conditions: the temperature of 23 ± 1 ℃, a 12h/12 h light/dark cycle (lighting from 08:00 to 20:00), and had free access to a sterile diet. The rats were then divided into 2groups. The first group of rats were designated as the control group (*n* = 25, normal and healthy) given PBS 2 ml/d per rat, and another group as the model group of PI-IBS (*n* = 85) filled with *C. jejuni* 1010 cfu/ml 2 ml/d per rat through an intra-gastric needle (Thermo Fisher Scientific, Hampton, US). The gavage continues for 7 d. Then the stool culture, body weight of the rat, stool water relative content would be test to evaluate the phase of infection.

If the rats get rid of infection with a higher Bristol score of faeces, faster intestinal transit and visceral hypersensitivity, then rats were considered to enter the post-infectious phase as PI-IBS.

After the model evaluation, the rats were regrouped, and the control group, designated as M (*n* = 20) filled with PBS 2 ml/d/per rat, and the PI-IBS model group, was divided into four groups, and was given PBS 2 ml/d/per rat, undiluted LGG-s 2 ml/d/per rat, double diluted LGG-s, triple diluted LGG-s 2 ml/d/per rat (A, B, C, D, respectively, *n* = 20) through a gavage needle[[34](#_ENREF_34)]. Rats were maintained in treatments for 1.0, 2.0, 3.0, 4.0 wk during the experiment. Five rats of each group were sacrificed; the colons and brains were removed for later use each week.

During all experiments, housing and diet conditions were the same for all groups. Rats infected with *C. jejuni* were housed in another room to prevent cross-contamination from infected to uninfected. The protocol was approved by the Animal Use and Care Committee of Tianjin Medical University.(Figure 1)

***Campylobacter gavage***

Before the infection, all rats received 1ml of 5% bicarbonate solution (w/v) via a ball-tipped inoculating needle to neutralize the gastric acid. 30 min later, *C. jejuni* in 2 ml of PBS was given to PI-IBS modeling group and 2 ml of sterile PBS to the control group[[37](#_ENREF_37)].

***Assessment of acute colonization by C. jejuni***

The fresh stool specimens were cultured for the presence of *C. jejuni* on Campylobacter selective agar plates (Biochemical tests, DNA agarose gel electrophoresis), and the general condition, weight, stool water relative content were observed or tested at the 3rd, 7th, 14th, 28th, 42nd, 56th, 70th, after gavage. The successful intestinal colonization of rat was defined by the detection of *C. jejuni* in stool at least once andclearance of infection was defined by two consecutive tests with negative culture.

Biochemical tests contain catalase test, oxidase test, hippurate hydrolysis test, and 3-indoylacetate hydrolysis test (GB 4789.9-2014, China). Total DNA was extracted using Campylobacter Nucleic Acid Test Kit (ZC-CAMPY-003, KangdaZhongchuang biotechnology co. LTD, China), tested with agarose gel electrophoresis.

For stool water relative content, fresh stools were obtained from rats under manual restraint by spontaneous or perianal-stimulated defecation, weighed, and then dried at 50℃ for 72 h followed by room temperature for 48 h. Dry stools were weighed again to determine the percent wet weight of stool.

***Determination of PI-IBS***

As two consecutive tests with negative culture, rats were considered to get rid of infection. Rats without infection were kept separately from infected rats. After all of the rats no longer had detectable *C. jejuni* in the stool, they were considered to be in the post-infectious time period. Fresh stool was collected for 3 consecutive days from all rats and graded by a modified Bristol Stool score[[38](#_ENREF_38)]. Normal stool was graded as 1; soft and poorly formed stool was graded as 2; and watery stool was graded as 3. Five from control group and four from model group were randomly chosen to get tests. The visceral hypersensitivity was evaluated by abdominal withdrawal reflex (AWR) test， and the intestinal motility was detected.

AWR experiment was done as previously reported[[39](#_ENREF_39)]. Rats were fasted for 18 h before the test was performed. The night before AWR, the balloons (7-8 mm diameter) were inflated overnight to stretch the latex, then the balloons became compliant. Following anesthesia with ether inhalation, the balloon coated with paraffin oil was inserted into the rectum with the tail of the balloon, 1 cm from the anus and fixed at the base of the tail. The balloon was connected *via* a double barreled cannula, one joint connected the air pump and another connected the pressure gauge. Rats were given 30 min to accommodate the environment. Then the balloon was distended with the pressures of 20, 40, 60, 80 mmHg. Distention was sustained for 15 s, at the intervals of 10 min. The distention was performed three times at each pressure, and the AWR scores were recorded. AWR was done by researchers who had no understanding of the experiment.

Intestinal motility was detected by activated carbon solution gavage. Before the experiment, rats were fasted for 24 h and then filled with 2 ml 10% activated carbon solution through an inoculating needle. After 50 min, rats were sacrificed by cervical dislocation. Then the laparotomy was performed and the whole bowel was taken out, moistened with PBS. The bowel was freely flattened on the table. The length of the bowel along which it contains the activated carbon and the length of the whole bowel were measured, and the ratio of these two lengths were taken as the intestinal transit rate (ITR)[[40](#_ENREF_40)].

***Real-time polymerase chain reaction***

To evaluate levels of SERT mRNA after treatment with LGG-s, samples of colon and brain were harvested. Rats were sacrificed by cervical dislocation, and 50 mg of the tissues from each rat was treated with Trizol, according to the manufacturer’s instructions (Life, Hilden, Germany). The iScriptcDNA synthesis kit (Bio-Rad Laboratories, Inc., Hercules, CA, United States) was used to synthesize the cDNA. The PCR were set up in a volume of 20 μl containing 1.0 μl cDNA, 10 μl 2 × iQSYBR Green Supermix (Roche Applied Science), and 0.6μl both forward and reverse primers, replenished with DEPC treated ddH2O. Quantitative real-time (QRT) PCR was performed on an ABI One plus setup PCR thermo-cycler. The primers for each sequence are given in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was measured as an internal control. Relative mRNA expression was calculated using the 2-ΔΔCt method.

***Western blotting***

Proteins were extracted from colonic and cerebral tissue, and levels were quantified using a BCA protein concentration assay kit (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), according to the manufacturer’s instructions. The protein samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membrane was blocked with 5% nonfat milk, and then incubated with primary antibody (SERT antibody: dilution 1:5000, EPR12735, Abcam Biotech Company, Cambridge, United Kingdom; β-actin: dilution 1:1000, 8457S, Cell Signaling Technology, Boston, United States) overnight at 4 ℃, and then incubated with secondary antibody (dilution 1:10000, BA1054, Boster Biological Technology Co., Ltd, Wuhan, China) for 1h at room temperature. The immunoreactive bands were visualized using an ECL Western Blotting Substrate (Solarbio Life Sciences Co., Ltd, Beijing, China) and exposure to X-ray film. β-actin was used as an inner control.

***Statistical analysis***

The statistical analysis was carried out using SPSS 22.0 (SPSS, Chicago, IL, USA). Quantitative data was expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) and post hoc tests (LSD and Dunnett’s T3) were used to compare the values of real-time polymerase chain reaction (RT-PCR). For all analyses, *P* < 0.05 was defined as statistically significance.

**RESULTS**

***Campylobacter colonization phase***

Total of 110 rats were used in the study, none showed *C. jejuni* infection prior to the first gavage (as determined by stool culture), and all rats inoculated with *C. jejuni* exhibited *C. jejuni* colonization within the 3rd day after gavage (1 rat died with severe gavage trauma). Thus, a total of 109 rats (25 control rats, 84 PI-IBS model rats) were included before the model evaluation.

3-14 d after infection, rats in control group were in good spirits, normal activity and glossy hair. However, the PI-IBS modeling group showed decadent, indolent and lackluster. The general conditions were returned to normal in about 42 d.

The weight in model group was significantly lower than that in control group at 3rd, 7th, 14th, 28th day (*P* < 0.05). Followed over time, the weight in model group was still lower than control with no statistical significance (*P* > 0.05).

The water relative content of the fresh stool in model group was significantly higher than control group at 3rd, 7th, 14th, 28th, 70th day (*P* < 0.05). At the 42th, and 56th day, the model group was a little higher than control group (Figure 2).

***Post-infectious phase***

As two consecutive tests resulted in negative culture, rats were considered to be rid of infection. At the 42th day, 95% of the *C. jejuni* rats were no longer infected. At the 56th and 70th day, all *C. jejuni* rats were test negatively. At the 70th day, tests were done to evaluate the PI-IBS. The 3-day average Bristol score of stool is higher in model group than that in control group, same as the intestinal transit rate (*P* < 0.05). AWR score of rats in model group was also higher than control group. However, there was no statistical difference between the two groups at the pressure of 20, 80 mmHg (*P* = 0.12; *P* = 0.06, respectively) (Figure 3).

***Effects of LGG-s on SERT mRNA and SERT-P expression in rat intestinal tissues***

For the PI-IBS PBS gavage group (A), the levels of SERT mRNA expression were lower than control group (M) throughout the experiment. However, only at the third week, A was under M by 0.7-fold (*P* = 0.023, *P* < 0.05).

Undiluted LGG-s (B) up-regulated SERT mRNA expression levels by 1.8-times the A by the first week (*P* = 0.002) and increased slightly higher than M (*P* > 0.05). By the end of the second week, the levels of B slowly rise to 2.67 times and 3.7 times M and A (*P* = 0.018, *P* = 0.012) respectively. It increased continuously at the third week by 4.4-fold, 6.1-fold and 1.7-fold more than M, A and D (Triple diluted LGG-s) (*P* = 0.003, *P* = 0.003, *P* = 0.028) respectively, and no significant difference with double diluted LGG-s (C). By the last week, the levels of SERT mRNA were only 2.1-fold than A (*P* = 0.016, *P* < 0.05).

Double diluted LGG-s (C) moderately increased the levels higher than A by 1.8-fold (*P* = 0.027). It increased like B at the second week by 2.3-fold (*P* = 0.012) compared to A, 4.6-and 6.4-fold at the third week, 1.9-and 2.6-fold at the last week compared to M and A (*P* = 0.012, *P* = 0.009, *P* = 0.032, *P* = 0.011, respectively). However, there was no statistical significant between C and D at the second week (*P* > 0.05).

Triple diluted LGG-s (D) significantly up-regulated SERT mRNA expression levels by 6.9-, 9.4-, 5.3-and 5.1-fold compared to M, A, B and C at the end of the first week (*P* = 0.02, *P* = 0.01, *P* = 0.002, *P* = 0.002, *P* < 0.05, respectively), but levels decreased rapidly at the end of the second week. The SERT expression levels were similar between the second week and the third week, 2.3-; 2.5-fold compared to M (*P* = 0.015; *P* = 0.001, *P* < 0.05) and 3.1-; 3.5-times the A (*P* = 0.009; *P* = 0.028, *P* < 0.05).

The variation tendency of SERT-P levels was similar to that of mRNA. A was lower than M during the whole experiment, and only the third week was with no significant difference (*P* = 0.177, *P* > 0.05). The levels of B were similar to A; lower than all other groups, at the first week. It was basically flat for B, C and D by the second and third week. It was a slight higher for B, C, D than M at the fourth week, with no statistical significance (Figure 4).

***Effects of LGG-s on SERT mRNA and SERT-P expression in rat brain tissues***

There were no statistical differences between the different dilution concentration throughout the experiment on SERT mRNA and SERT-P expression. The levels of SERT mRNA in B, C and D had a little increase compared to M and A at the first week, but turned lower at the last week. Double diluted LGG-s showed a minuscule growth of SERT-P in the third week, and went down at the end of the study (Figure 5).

**DISCUSSION**

According to the Rome IV criteria, IBS is one of the lower gastrointestinal tract disorders, which identify about two thirds of suffers, along with requiring that the pain either be relieved by defecation or associated with changes in stool frequency or/and consistency[[8](#_ENREF_8),[41](#_ENREF_41),[42](#_ENREF_42)]. PI-IBS often exhibits characteristics of diarrhea, which has a history of acute gastrointestinal infection[[9-12](#_ENREF_9)]. Many studies showed that infecting *C. jejuni* strain correlates with the development of PI-IBS[[43-45](#_ENREF_43)]. *C. jejuni* produces a range of toxins including cytolethal distending toxin, which first produces a secretory diarrhea in the small intestine, inducing an invasion of the distal ileum and colon to produce an inflammatory ileocolitis[[46](#_ENREF_46)]. Therefore, *C. jejuni* was used to build the model of PI-IBS in this study. The mechanisms that underlie chronic disturbance of gut function are thought to involve chronic mucosal microscopic inflammation, visceral hypersensitivity, dysregulation of gut microbiota and abnormal neuromuscular function[[47-50](#_ENREF_47)].

Serotonin was found in the gastrointestinal (GI) tract and central nervous system (CNS). It showed to function both as a neurotransmitter and as local hormone in the peripheral vascular system in the gut[[51](#_ENREF_51)]. About 95% of body 5-HT is found in the gastrointestinal tract, 90% in enterochromaffin cells (EC cell) and 10% in serotonergic neurons of myenteric plexus[[51](#_ENREF_51),[52](#_ENREF_52)]. As a signal transducer and a neurotransmitter, 5-HT is a key molecule, regulating visceral perception and intestinal motility, and most of the 5-HT in the body is in the gut[[53](#_ENREF_53)]. Serotonin exerts its action by binding to its receptors (5-HT1 to 5-HT7) present in both intrinsic and extrinsic primary afferent neurons[[54](#_ENREF_54)]. Serotonin receptors that are known to affect gut motor functions are those belonging to the 5-HT1, 2, 3, 4, 7 subtypes[[55-57](#_ENREF_55)]. 5-HT receptors contract effector cells by 5-HT2A and relax cells by 5-HT4, 5-HT7 subtypes[[58](#_ENREF_58)]. Neuronal 5-HT3 receptor leads to increased release of acetylcholine (Ach) from cholinergic neurons[[52](#_ENREF_52)]. The release of 5-HT acting on effector cells leading to secretory reflexes, peristaltic reflexes, and if superfluous, diarrhea and abdominal pain or visceral hypersensitivity[[59](#_ENREF_59)]. Clinical trials have shown an increased level of 5-HT in IBS[[60-62](#_ENREF_60)]. Serotonin reuptake transporter (SERT) plays an irreplaceable role in 5-HT inactivation by decreasing the content of serotonin in synaptic cleft[[63](#_ENREF_63)].

SERT on the cell membrane of enterocytes are vital to transport 5-HT intracellular, with 5-HT metabolized by monoamine oxidase[[64](#_ENREF_64)]. SERT was expressed by nearly all of the intestinal epithelial cells on the surface of the lumen[[65](#_ENREF_65)]. Biochemical abnormalities in PI-IBS patients including hyperplasia of the EC cells and depressed 5-hydroxyindole acetic acid/5-HT ratios, suggested an impaired SERT function[[44](#_ENREF_44)]. Many researchers have demonstrated that IBS patients have a remarkably lower level of SERT expression in the intestine[[66](#_ENREF_66)]. Coates *et al*[[17](#_ENREF_17)] first exhibited a significantly decreased level of SERT in IBS.

Regulation of SERT expression contains a series of factors, such as gene polymorphisms, micro RNAs, immunity, inflammation, gut microbiota and growth factors[[67](#_ENREF_67)]. (1) The SERT gene, solute carrier family 6 member 4 (SLC6A4) has a positive association with etiology of IBS, including 5-HT-transportergene-linked polymorphic region (5-HTTLPR)[[19](#_ENREF_19)], a variable number of tandem repeats (VNTR) STin2[[68](#_ENREF_68)], and functional single nucleotide polymorphisms (SNPs)[[69](#_ENREF_69)]. 5-HTTLPR is the most frequently studied, subdivided into long (L) and short (S) alleles[[19](#_ENREF_19)]. Previous studies found that the L/L genotype is significantly higher in C-IBS than L/S and S/S[[21](#_ENREF_21)]. Variable number of tandem repeats (VNTR) showed a higher ratio in STin2.12/10 and a lower ratio in STin2.12/12 in IBS[[25](#_ENREF_25)]. (2) Immune activation of the gut mucosa plays a critical role in EC cell hyperplasia and reduced SERT activity in PI-IBS[[70](#_ENREF_70)]. Foley *et al*[[71](#_ENREF_71)] found SERT mRNA preformed a lower level in D-IBS, correlated with increased numbers of mucosal intraepithelial lymphocytes (IELs) and mast cells. Pro-inflammatory mediators, such as interferon-γ (IFN-γ) and tumor necrosis factor –α (TNF-α), reduce the expression of SERT mRNA, SERT-P and SERT function in Caco-2 cells[[72](#_ENREF_72)]. However, a protective cytokine, transforming growth factor-β1 (TGF-β1) could rapidly activate SERT activity and inhibit intestinal inﬂammation by PI3K and syntaxin 3[[73](#_ENREF_73)]. The acute infection of *C. jejuni* produces diarrhea in the small intestine, and leads to inflammatory response, which increases the ratio of pro-/anti-inflammatory factors. In other words, the SERT mRNA and SERT-P levels in intestinal tissues were significantly decreased in PI-IBS PBS group than control. With the treatment of LGG-s, the highest concentration did not induce a highest expression of SERT, by contrast with previous study which found a dose-response of expression of SERT[34]. This may due to the differences in the contents of various substances (proteins, fatty acids, inorganic salts, *etc*.) in the supernatant, conbined with previous *C. jejuni* infection, which could lead to different immune activation. (3) Gut host-microbial interactions are important factors in the IBS. Studies have found that *Lactobacillus*, *Bifidobacterium*, *Actinobacteria* and *Bacteroidetes* were decreased[[74-76](#_ENREF_74)], while *Proteobacteria*, *Firmicutes* and *Firmicutes*/*Bacteroidetes* ratios were increased in fecal samples of IBS-D patients[[77](#_ENREF_77)]. Enteropathogenic *E. coli* and *E. coli* Nissle 1917 could decrease SERT mRNA and increase 5-HT bioavailability[[78](#_ENREF_78),[79](#_ENREF_79)]. Our previous study proved that LGG-s could up-regulated the SERT mRNA and SERT-P levels in enterocytes and mouse intestinal tissues[[34](#_ENREF_34)]. In this study, we also proved that LGG-s also have positive affection on SERT expression in colon tissues in PI-IBS rats. It may provide a novel solution for the treatment of IBS. Further researches are needed to find whether LGG-s has any impact on the composition of rat gut microbiota. (4) Growth factors, such as epidermal growth factor, basic fibroblast growth factor, and nerve growth factor found evidences in the up-regulation of SERT expression[[80-82](#_ENREF_80)]. A protein, known as p40, expressed by LGG, activates the epidermal growth factor receptor (EGFR)[[35](#_ENREF_35)] and, might also activate the mechanism of LGG-s up-regulated SERT expression. And (5) Gender may be another factor. Although the IBS is an universally disease, women are more likely to suffer from illness than men[[83](#_ENREF_83)]. They also found that SERT mRNA levels in the rectal mucosa of women with IBS-D were higher than those in men. Female SERT KO rats showed a remarkable visceral hypersensitivity than male[84]. However, male animals for PI-IBS study were also common in the research[85,86]. Ibeakanma *et al*[87] found that brain-gut interactions could increase a exaggerated peripheral nociceptive signaling in male mice with PI-IBS. An experimental model of male mouse induced by stress or Giardia also showed a prominent visceral hypersensitivity[88]. As for this study, male rats were selected to make a research.

Alterations in the bidirectional interactions between the gut and the nervous system play an important role in IBS pathophysiology and symptom generation. Communication between the gut and the central nervous system, both in the ascending (gut-to-brain) and descending (brain-to-gut) directions is called the gut–brain axis[[89](#_ENREF_83)]. Gut microbes may communicate with the gut–brain axis via production of neuro-active and neuroendocrine molecules such as serotonin, aminobutyric acid (GABA), histamine, noradrenaline and adrenaline[[90](#_ENREF_84)]. *Lactobacilli* can convert glutamate into GABA[[91](#_ENREF_85)], and given *L. rhamnosus* JB-1 to mice altered patterns of GABA receptors in the brain[92]. There is very little study about the changes of SERT in the CNS. While, no significant differences were found between the LGG-s treated group and the control group in this study. It is possible that the signal might be prevented from entering the brain by the blood-brain barrier (BBB)[[93](#_ENREF_87)], and thereby not causing alteration in the task. The connections between cells in BBB are tighter, which greatly limits the endothelial permeability *via* para-cellular and trans-cellular transport pathways[94]. The exchange of substances in the blood and brain is mainly accomplished by various transporters expressed in vascular endothelial cells[[95](#_ENREF_89)]. Regulators or neurotransmitters, secreted by LGG, may lack specific transporters. Because of the BBB, it is very difficult for 5-HT in the blood to enter the CNS, whereas 5-HT of the central and peripheral nerves is a separate system with different functions. Therefore, as the reuptake transporter, SERT may also be regulated by different passageways. The effect of LGG injected directly into the brain is still need to be studied.

In conclusion, we found that LGG-s up-regulate SERT expression in intestinal tissues, but have no statistical difference in brain tissues in PI-IBS rats. By decreasing 5-HT levels, LGG-s may be a potential method of assisting IBS.

**Article Highlights**

***Research background***

The probiotics are approved to be used to relieve irritable-bowel syndrome (IBS), and *Lactobacillus rhamnosus* GG (LGG) is the best studied member of lactic acid bacteria play a supportive therapeutic efficacy in IBS. However, the mechanism is still remains a significant challenge to researchers. This study explores that pathway by building a PI-IBS model, evaluating the effect of LGG supernatant on serotonin transporter.

***Research motivation***

This study is a part of National Natural Science Foundation of China project. On the basis of experimental model of PI-IBS rats, the research explores the mechanism of LGG-s’ effect on SERT levels in intestinal and brain tissues.

***Research objectives***

The study tests the expression levels of SERT mRNA and SERT-protein to evaluate the affection of LGG-s in the PI-IBS rats, which infected with *C. jejuni*. LGG-s could up-regulate SERT mRNA and SERT-P levels in rat intestinal tissues and has no influence in rat brain tissues. The use of this PI-IBS model will support a better study, and the more detailed research on LGG-s will contribute to more accurate treatment of IBS.

***Research methods***

The model group of PI-IBS (*n* = 85) filled with *C. jejuni* 1010 cfu/ml 2 ml/d per rat, continuing 7 d, then the body weight of the rat, stool water relative content would be test to evaluate the phase of infection, and the fresh stool specimens were cultured for the presence of *C. jejuni* on *Campylobacter* selective agar plates. After the model evaluation, the rats were regrouped, each group was gavaged with different concentration of LGG-s. Rats were maintained in treatments for 1.0, 2.0, 3.0, 4.0 wk during the experiment. Then, SERT would be test by RT-PCR and Western blotting to evaluate the affection of LGG-s.

***Research results***

The levels of SERT-mRNA and SERT-P in the intestinal tissues were up-regulated in the treatment of LGG-s, although with the different concentrations. Triple-diluted LGG-s showed a more significant difference within a short term, while, in the long run, undiluted and double-diluted LGG-s proved better. However, there were no significant differences found for SERT-mRNA and SERT-P in the brain tissues between each group, with or without treatment of LGG-s. Some factors, differences in the contents of various substances (proteins, fatty acids, inorganic salts, *etc*.) in the supernatant, may induced these different increases of SERT levels. More details researches about LGG-s were needed.

***Research conclusions***

The study has demonstrated that LGG-s up-regulate SERT expression in intestinal tissues, but have no statistical difference in brain tissues in PI-IBS rats. The previous study has proved that LGG-s could up-regulate the SERT levels in intestinal tissues in healthy mice. Moreover, the concentration of LGG-s leaded to a dose-response of expression of SERT. The contents of substances in the supernatant, combined with their different concentration, molecular mass, and previous *C. jejuni* infection of rats, may result in this phenomenon. Therefore, more details researches about LGG-s and relief of clinical symptoms with the treatment of LGG-s would be done in our next work.

***Research perspectives***

The infection with *C. jejuni* could help to build a PI-IBS model with a lower expression of SERT. For the future accurate treatment of IBS, proteomics analysis of LGG-s was an important and urgent research.

**ACKNOWLEDGMENTS**

We thank our laboratory counselors, Jing-Wen Zhao and Wei-Qiang Wang, for their valuable support and guidance in the research work.

**REFERENCES**

1 **Drossman DA**, Hasler WL. Rome IV-Functional GI Disorders: Disorders of Gut-Brain Interaction. *Gastroenterology* 2016; **150**: 1257-1261 [PMID: 27147121 DOI: 10.1053/j.gastro.2016.03.035]

2 **Kim DY**, Camilleri M. Serotonin: a mediator of the brain-gut connection. *Am J Gastroenterol* 2000; **95**: 2698-2709 [PMID: 11051338 DOI: 10.1111/j.1572-0241.2000.03177.x]

3 **Drossman DA**, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology* 2002; **123**: 2108-2131 [PMID: 12454866 DOI: 10.1053/gast.2002.37095]

4 **Porter CK**, Faix DJ, Shiau D, Espiritu J, Espinosa BJ, Riddle MS. Postinfectious gastrointestinal disorders following norovirus outbreaks. *Clin Infect Dis* 2012; **55**: 915-922 [PMID: 22715178 DOI: 10.1093/cid/cis576]

5 **Xiong LS**, Chen MH, Chen HX, Xu AG, Wang WA, Hu PJ. A population-based epidemiologic study of irritable bowel syndrome in South China: stratified randomized study by cluster sampling. *Aliment Pharmacol Ther* 2004; **19**: 1217-1224 [PMID: 15153175 DOI: 10.1111/j.1365-2036.2004.01939.x]

6 **Longstreth GF**, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; **130**: 1480-1491 [PMID: 16678561 DOI: 10.1053/j.gastro.2005.11.061]

7 **Engsbro AL**, Simren M, Bytzer P. Short-term stability of subtypes in the irritable bowel syndrome: prospective evaluation using the Rome III classification. *Aliment Pharmacol Ther* 2012; **35**: 350-359 [PMID: 22176384 DOI: 10.1111/j.1365-2036.2011.04948.x]

8 **Ford AC**, Bercik P, Morgan DG, Bolino C, Pintos-Sanchez MI, Moayyedi P. Validation of the Rome III criteria for the diagnosis of irritable bowel syndrome in secondary care. *Gastroenterology* 2013; **145**: 1262-70.e1 [PMID: 23994201 DOI: 10.1053/j.gastro.2013.08.048]

9 **Marshall JK**, Thabane M, Garg AX, Clark WF, Moayyedi P, Collins SM; Walkerton Health Study Investigators. Eight year prognosis of postinfectious irritable bowel syndrome following waterborne bacterial dysentery. *Gut* 2010; **59**: 605-611 [PMID: 20427395 DOI: 10.1136/gut.2009.202234]

10 **Marshall JK**, Thabane M, Garg AX, Clark WF, Salvadori M, Collins SM; Walkerton Health Study Investigators. Incidence and epidemiology of irritable bowel syndrome after a large waterborne outbreak of bacterial dysentery. *Gastroenterology* 2006; **131**: 445-50; quiz 660 [PMID: 16890598 DOI: 10.1053/j.gastro.2006.05.053]

11 **Thabane M**, Kottachchi DT, Marshall JK. Systematic review and meta-analysis: The incidence and prognosis of post-infectious irritable bowel syndrome. *Aliment Pharmacol Ther* 2007; **26**: 535-544 [PMID: 17661757 DOI: 10.1111/j.1365-2036.2007.03399.x]

12 **Andresen V**, Löwe B, Broicher W, Riegel B, Fraedrich K, von Wulffen M, Gappmayer K, Wegscheider K, Treszl A, Rose M, Layer P, Lohse AW. Post-infectious irritable bowel syndrome (PI-IBS) after infection with Shiga-like toxin-producing Escherichia coli (STEC) O104:H4: A cohort study with prospective follow-up. *United European Gastroenterol J* 2016; **4**: 121-131 [PMID: 26966532 DOI: 10.1177/2050640615581113]

13 **Yan C**, Xin-Guang L, Hua-Hong W, Jun-Xia L, Yi-Xuan L. Effect of the 5-HT4 receptor and serotonin transporter on visceral hypersensitivity in rats. *Braz J Med Biol Res* 2012; **45**: 948-954 [PMID: 22832600]

14 **Zang KH**, Shao YY, Zuo X, Rao Z, Qin HY. Oridonin Alleviates Visceral Hyperalgesia in a Rat Model of Postinflammatory Irritable Bowel Syndrome: Role of Colonic Enterochromaffin Cell and Serotonin Availability. *J Med Food* 2016; **19**: 586-592 [PMID: 27111743 DOI: 10.1089/jmf.2015.3595]

15 **Dizdar V**, Spiller R, Singh G, Hanevik K, Gilja OH, El-Salhy M, Hausken T. Relative importance of abnormalities of CCK and 5-HT (serotonin) in Giardia-induced post-infectious irritable bowel syndrome and functional dyspepsia. *Aliment Pharmacol Ther* 2010; **31**: 883-891 [PMID: 20132151]

16 **Barbara G**, Cremon C. Serine proteases: new players in diarrhoea-predominant irritable bowel syndrome. *Gut* 2008; **57**: 1035-1037 [PMID: 18628370 DOI: 10.1136/gut.2008.150821]

17 **Coates MD**, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM, Moses PL. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. *Gastroenterology* 2004; **126**: 1657-1664 [PMID: 15188158]

18 **Lesch KP**, Balling U, Gross J, Strauss K, Wolozin BL, Murphy DL, Riederer P. Organization of the human serotonin transporter gene. *J Neural Transm Gen Sect* 1994; **95**: 157-162 [PMID: 7865169]

19 **Lesch KP**, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996; **274**: 1527-1531 [PMID: 8929413]

20 **Hu XZ**, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, Xu K, Arnold PD, Richter MA, Kennedy JL, Murphy DL, Goldman D. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. *Am J Hum Genet* 2006; **78**: 815-826 [PMID: 16642437 DOI: 10.1086/503850]

21 **Wang YM**, Chang Y, Chang YY, Cheng J, Li J, Wang T, Zhang QY, Liang DC, Sun B, Wang BM. Serotonin transporter gene promoter region polymorphisms and serotonin transporter expression in the colonic mucosa of irritable bowel syndrome patients. *Neurogastroenterol Motil* 2012; **24**: 560-565, e254-e255 [PMID: 22435794 DOI: 10.1111/j.1365-2982.2012.01902.x]

22 **Choi YJ**, Hwang SW, Kim N, Park JH, Oh JC, Lee DH. Association Between SLC6A4 Serotonin Transporter Gene Lainked Polymorphic Region and ADRA2A -1291C&gt;G and Irritable Bowel Syndrome in Korea. *J Neurogastroenterol Motil* 2014; **20**: 388-399 [PMID: 24917480 DOI: 10.5056/jnm14020]

23 **Zhang ZF**, Duan ZJ, Wang LX, Yang D, Zhao G, Zhang L. The serotonin transporter gene polymorphism (5-HTTLPR) and irritable bowel syndrome: a meta-analysis of 25 studies. *BMC Gastroenterol* 2014; **14**: 23 [PMID: 24512255 DOI: 10.1186/1471-230x-14-23]

24 **Colucci R**, Gambaccini D, Ghisu N, Rossi G, Costa F, Tuccori M, De Bortoli N, Fornai M, Antonioli L, Ricchiuti A, Mumolo MG, Marchi S, Blandizzi C, Bellini M. Influence of the serotonin transporter 5HTTLPR polymorphism on symptom severity in irritable bowel syndrome. *PLoS One* 2013; **8**: e54831 [PMID: 23393559 DOI: 10.1371/journal.pone.0054831]

25 **Chen ZR**, Qian GB, Wu ZX. [Cryptogenetic multifocal ulcerous stenosing enteritis]. *Zhonghua Nei Ke Za Zhi* 2017; **56**: 618-620 [PMID: 28789501 DOI: 10.3760/cma.j.issn.0578-1426.2017.08.017]

26 **Wheatcroft J**, Wakelin D, Smith A, Mahoney CR, Mawe G, Spiller R. Enterochromaffin cell hyperplasia and decreased serotonin transporter in a mouse model of postinfectious bowel dysfunction. *Neurogastroenterol Motil* 2005; **17**: 863-870 [PMID: 16336502 DOI: 10.1111/j.1365-2982.2005.00719.x]

27 **Floch MH**. Recommendations for probiotic use in humans-a 2014 update. *Pharmaceuticals (Basel)* 2014; **7**: 999-1007 [PMID: 25310351 DOI: 10.3390/ph7100999]

28 **McFarland LV**, Dublin S. Meta-analysis of probiotics for the treatment of irritable bowel syndrome. *World J Gastroenterol* 2008; **14**: 2650-2661 [PMID: 18461650]

29 **Yang GY**, Yu J, Su JH, Jiao LG, Liu X, Zhu YH. Oral Administration of Lactobacillus rhamnosus GG Ameliorates Salmonella Infantis-Induced Inflammation in a Pig Model via Activation of the IL-22BP/IL-22/STAT3 Pathway. *Front Cell Infect Microbiol* 2017; **7**: 323 [PMID: 28770173 DOI: 10.3389/fcimb.2017.00323]

30 **Jiang Y**, Ye L, Cui Y, Yang G, Yang W, Wang J, Hu J, Gu W, Shi C, Huang H, Wang C. Effects of Lactobacillus rhamnosus GG on the maturation and differentiation of dendritic cells in rotavirus-infected mice. *Benef Microbes* 2017; **8**: 645-656 [PMID: 28670908 DOI: 10.3920/bm2016.0157]

31 **Hojsak I**, Szajewska H, Canani RB, Guarino A, Indrio F, Kolacek S, Orel R, Shamir R, Vandenplas Y, van Goudoever JB, Weizman Z; ESPGHAN Working Group for Probiotics/Prebiotics. Probiotics for the Prevention of Nosocomial Diarrhea in Children. *J Pediatr Gastroenterol Nutr* 2017; Epub ahead of print [PMID: 28574970 DOI: 10.1097/mpg.0000000000001637]

32 **Francavilla R**, Miniello V, Magistà AM, De Canio A, Bucci N, Gagliardi F, Lionetti E, Castellaneta S, Polimeno L, Peccarisi L, Indrio F, Cavallo L. A randomized controlled trial of Lactobacillus GG in children with functional abdominal pain. *Pediatrics* 2010; **126**: e1445-e1452 [PMID: 21078735 DOI: 10.1542/peds.2010-0467]

33 **Pedersen N**, Andersen NN, Végh Z, Jensen L, Ankersen DV, Felding M, Simonsen MH, Burisch J, Munkholm P. Ehealth: low FODMAP diet vs Lactobacillus rhamnosus GG in irritable bowel syndrome. *World J Gastroenterol* 2014; **20**: 16215-16226 [PMID: 25473176 DOI: 10.3748/wjg.v20.i43.16215]

34 **Wang YM**, Ge XZ, Wang WQ, Wang T, Cao HL, Wang BL, Wang BM. Lactobacillus rhamnosus GG supernatant upregulates serotonin transporter expression in intestinal epithelial cells and mice intestinal tissues. *Neurogastroenterol Motil* 2015; **27**: 1239-1248 [PMID: 26088715 DOI: 10.1111/nmo.12615]

35 **Yan F**, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology* 2007; **132**: 562-575 [PMID: 17258729 DOI: 10.1053/j.gastro.2006.11.022]

36 **Pimentel M**, Chatterjee S, Chang C, Low K, Song Y, Liu C, Morales W, Ali L, Lezcano S, Conklin J, Finegold S. A new rat model links two contemporary theories in irritable bowel syndrome. *Dig Dis Sci* 2008; **53**: 982-989 [PMID: 17934822 DOI: 10.1007/s10620-007-9977-z]

37 **Jee SR**, Morales W, Low K, Chang C, Zhu A, Pokkunuri V, Chatterjee S, Soffer E, Conklin JL, Pimentel M. ICC density predicts bacterial overgrowth in a rat model of post-infectious IBS. *World J Gastroenterol* 2010; **16**: 3680-3686 [PMID: 20677340 DOI: 10.3748/wjg.v16.i29.3680]

38 **Pokkunuri V**, Pimentel M, Morales W, Jee SR, Alpern J, Weitsman S, Marsh Z, Low K, Hwang L, Khoshini R, Barlow GM, Wang H, Chang C. Role of Cytolethal Distending Toxin in Altered Stool Form and Bowel Phenotypes in a Rat Model of Post-infectious Irritable Bowel Syndrome. *J Neurogastroenterol Motil* 2012; **18**: 434-442 [PMID: 23106005 DOI: 10.5056/jnm.2012.18.4.434]

39 **Al-Chaer ED**, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. *Gastroenterology* 2000; **119**: 1276-1285 [PMID: 11054385]

40 **Wang W**, Xin H, Fang X, Dou H, Liu F, Huang D, Han S, Fei G, Zhu L, Zha S, Zhang H, Ke M. Isomalto-oligosaccharides ameliorate visceral hyperalgesia with repair damage of ileal epithelial ultrastructure in rats. *PLoS One* 2017; **12**: e0175276 [PMID: 28437458 DOI: 10.1371/journal.pone.0175276]

41 **Schmulson MJ**, Drossman DA. What Is New in Rome IV. *J Neurogastroenterol Motil* 2017; **23**: 151-163 [PMID: 28274109 DOI: 10.5056/jnm16214]

42 **Spiller R**. Clinical update: irritable bowel syndrome. *Lancet* 2007; **369**: 1586-1588 [PMID: 17499587 DOI: 10.1016/s0140-6736(07)60726-0]

43 **Thornley JP**, Jenkins D, Neal K, Wright T, Brough J, Spiller RC. Relationship of Campylobacter toxigenicity in vitro to the development of postinfectious irritable bowel syndrome. *J Infect Dis* 2001; **184**: 606-609 [PMID: 11474430 DOI: 10.1086/322845]

44 **Dunlop SP**, Jenkins D, Neal KR, Spiller RC. Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. *Gastroenterology* 2003; **125**: 1651-1659 [PMID: 14724817]

45 **Swan C**, Duroudier NP, Campbell E, Zaitoun A, Hastings M, Dukes GE, Cox J, Kelly FM, Wilde J, Lennon MG, Neal KR, Whorwell PJ, Hall IP, Spiller RC. Identifying and testing candidate genetic polymorphisms in the irritable bowel syndrome (IBS): association with TNFSF15 and TNFα. *Gut* 2013; **62**: 985-994 [PMID: 22684480 DOI: 10.1136/gutjnl-2011-301213]

46 **Whitehouse CA**, Balbo PB, Pesci EC, Cottle DL, Mirabito PM, Pickett CL. Campylobacter jejuni cytolethal distending toxin causes a G2-phase cell cycle block. *Infect Immun* 1998; **66**: 1934-1940 [PMID: 9573072]

47 **Ford AC**, Talley NJ. Mucosal inflammation as a potential etiological factor in irritable bowel syndrome: a systematic review. *J Gastroenterol* 2011; **46**: 421-431 [PMID: 21331765 DOI: 10.1007/s00535-011-0379-9]

48 **Spiller R**, Garsed K. Postinfectious irritable bowel syndrome. *Gastroenterology* 2009; **136**: 1979-1988 [PMID: 19457422 DOI: 10.1053/j.gastro.2009.02.074]

49 **Saulnier DM**, Riehle K, Mistretta TA, Diaz MA, Mandal D, Raza S, Weidler EM, Qin X, Coarfa C, Milosavljevic A, Petrosino JF, Highlander S, Gibbs R, Lynch SV, Shulman RJ, Versalovic J. Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. *Gastroenterology* 2011; **141**: 1782-1791 [PMID: 21741921 DOI: 10.1053/j.gastro.2011.06.072]

50 **Jeffery IB**, O'Toole PW, Öhman L, Claesson MJ, Deane J, Quigley EM, Simrén M. An irritable bowel syndrome subtype defined by species-specific alterations in faecal microbiota. *Gut* 2012; **61**: 997-1006 [PMID: 22180058 DOI: 10.1136/gutjnl-2011-301501]

51 **Gershon MD**, Drakontides AB, Ross LL. Serotonin: synthesis and release from the myenteric plexus of the mouse intestine. *Science* 1965; **149**: 197-199 [PMID: 14305120]

52 **Sikander A**, Rana SV, Prasad KK. Role of serotonin in gastrointestinal motility and irritable bowel syndrome. *Clin Chim Acta* 2009; **403**: 47-55 [PMID: 19361459 DOI: 10.1016/j.cca.2009.01.028]

53 **Cremon C**, Carini G, Wang B, Vasina V, Cogliandro RF, De Giorgio R, Stanghellini V, Grundy D, Tonini M, De Ponti F, Corinaldesi R, Barbara G. Intestinal serotonin release, sensory neuron activation, and abdominal pain in irritable bowel syndrome. *Am J Gastroenterol* 2011; **106**: 1290-1298 [PMID: 21427712 DOI: 10.1038/ajg.2011.86]

54 **Barnes NM**, Sharp T. A review of central 5-HT receptors and their function. *Neuropharmacology* 1999; **38**: 1083-1152 [PMID: 10462127]

55 **Read NW**, Gwee KA. The importance of 5-hydroxytryptamine receptors in the gut. *Pharmacol Ther* 1994; **62**: 159-173 [PMID: 7991641]

56 **Galligan JJ**. Electrophysiological studies of 5-hydroxytryptamine receptors on enteric neurons. *Behav Brain Res* 1996; **73**: 199-201 [PMID: 8788502]

57 **Prins NH**, Briejer MR, Van Bergen PJ, Akkermans LM, Schuurkes JA. Evidence for 5-HT7 receptors mediating relaxation of human colonic circular smooth muscle. *Br J Pharmacol* 1999; **128**: 849-852 [PMID: 10556917 DOI: 10.1038/sj.bjp.0702762]

58 **Kuemmerle JF**, Murthy KS, Grider JR, Martin DC, Makhlouf GM. Coexpression of 5-HT2A and 5-HT4 receptors coupled to distinct signaling pathways in human intestinal muscle cells. *Gastroenterology* 1995; **109**: 1791-1800 [PMID: 7498643]

59 **Spiller R**. Serotonin and GI clinical disorders. *Neuropharmacology* 2008; **55**: 1072-1080 [PMID: 18687345 DOI: 10.1016/j.neuropharm.2008.07.016]

60 **Keszthelyi D**, Troost FJ, Jonkers DM, van Eijk HM, Dekker J, Buurman WA, Masclee AA. Visceral hypersensitivity in irritable bowel syndrome: evidence for involvement of serotonin metabolism--a preliminary study. *Neurogastroenterol Motil* 2015; **27**: 1127-1137 [PMID: 26031193 DOI: 10.1111/nmo.12600]

61 **Zhao JM**, Lu JH, Yin XJ, Chen XK, Chen YH, Tang WJ, Jin XM, Wu LY, Bao CH, Wu HG, Shi Y. Comparison of electroacupuncture and moxibustion on brain-gut function in patients with diarrhea-predominant irritable bowel syndrome: A randomized controlled trial. *Chin J Integr Med* 2015; **21**: 855-865 [PMID: 25847778 DOI: 10.1007/s11655-015-2049-x]

62 **Keszthelyi D**, Troost FJ, Jonkers DM, van Eijk HM, Lindsey PJ, Dekker J, Buurman WA, Masclee AA. Serotonergic reinforcement of intestinal barrier function is impaired in irritable bowel syndrome. *Aliment Pharmacol Ther* 2014; **40**: 392-402 [PMID: 24943480 DOI: 10.1111/apt.12842]

63 **Bjerregaard H**, Severinsen K, Said S, Wiborg O, Sinning S. A dualistic conformational response to substrate binding in the human serotonin transporter reveals a high affinity state for serotonin. *J Biol Chem* 2015; **290**: 7747-7755 [PMID: 25614630 DOI: 10.1074/jbc.M114.573477]

64 **Keating C**, Beyak M, Foley S, Singh G, Marsden C, Spiller R, Grundy D. Afferent hypersensitivity in a mouse model of post-inflammatory gut dysfunction: role of altered serotonin metabolism. *J Physiol* 2008; **586**: 4517-4530 [PMID: 18653657 DOI: 10.1113/jphysiol.2008.156984]

65 **Chen JJ**, Li Z, Pan H, Murphy DL, Tamir H, Koepsell H, Gershon MD. Maintenance of serotonin in the intestinal mucosa and ganglia of mice that lack the high-affinity serotonin transporter: Abnormal intestinal motility and the expression of cation transporters. *J Neurosci* 2001; **21**: 6348-6361 [PMID: 11487658]

66 **Faure C**, Patey N, Gauthier C, Brooks EM, Mawe GM. Serotonin signaling is altered in irritable bowel syndrome with diarrhea but not in functional dyspepsia in pediatric age patients. *Gastroenterology* 2010; **139**: 249-258 [PMID: 20303355 DOI: 10.1053/j.gastro.2010.03.032]

67 **Jin DC**, Cao HL, Xu MQ, Wang SN, Wang YM, Yan F, Wang BM. Regulation of the serotonin transporter in the pathogenesis of irritable bowel syndrome. *World J Gastroenterol* 2016; **22**: 8137-8148 [PMID: 27688655 DOI: 10.3748/wjg.v22.i36.8137]

68 **MacKenzie A**, Quinn J. A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. *Proc Natl Acad Sci U S A* 1999; **96**: 15251-15255 [PMID: 10611371]

69 **Yuan J**, Kang C, Wang M, Wang Q, Li P, Liu H, Hou Y, Su P, Yang F, Wei Y, Yang J. Association study of serotonin transporter SLC6A4 gene with Chinese Han irritable bowel syndrome. *PLoS One* 2014; **9**: e84414 [PMID: 24392134 DOI: 10.1371/journal.pone.0084414]

70 **Spiller R**, Lam C. An Update on Post-infectious Irritable Bowel Syndrome: Role of Genetics, Immune Activation, Serotonin and Altered Microbiome. *J Neurogastroenterol Motil* 2012; **18**: 258-268 [PMID: 22837873 DOI: 10.5056/jnm.2012.18.3.258]

71 **Kerckhoffs AP**, ter Linde JJ, Akkermans LM, Samsom M. SERT and TPH-1 mRNA expression are reduced in irritable bowel syndrome patients regardless of visceral sensitivity state in large intestine. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G1053-G1060 [PMID: 22323131 DOI: 10.1152/ajpgi.00153.2011]

72 **Foley KF**, Pantano C, Ciolino A, Mawe GM. IFN-gamma and TNF-alpha decrease serotonin transporter function and expression in Caco2 cells. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G779-G784 [PMID: 17170025 DOI: 10.1152/ajpgi.00470.2006]

73 **Nazir S**, Kumar A, Chatterjee I, Anbazhagan AN, Gujral T, Priyamvada S, Saksena S, Alrefai WA, Dudeja PK, Gill RK. Mechanisms of Intestinal Serotonin Transporter (SERT) Upregulation by TGF-β1 Induced Non-Smad Pathways. *PLoS One* 2015; **10**: e0120447 [PMID: 25954931 DOI: 10.1371/journal.pone.0120447]

74 **Simrén M**, Barbara G, Flint HJ, Spiegel BM, Spiller RC, Vanner S, Verdu EF, Whorwell PJ, Zoetendal EG; Rome Foundation Committee. Intestinal microbiota in functional bowel disorders: a Rome foundation report. *Gut* 2013; **62**: 159-176 [PMID: 22730468 DOI: 10.1136/gutjnl-2012-302167]

75 **Mayer EA**, Savidge T, Shulman RJ. Brain-gut microbiome interactions and functional bowel disorders. *Gastroenterology* 2014; **146**: 1500-1512 [PMID: 24583088 DOI: 10.1053/j.gastro.2014.02.037]

76 **Krogius-Kurikka L**, Lyra A, Malinen E, Aarnikunnas J, Tuimala J, Paulin L, Mäkivuokko H, Kajander K, Palva A. Microbial community analysis reveals high level phylogenetic alterations in the overall gastrointestinal microbiota of diarrhoea-predominant irritable bowel syndrome sufferers. *BMC Gastroenterol* 2009; **9**: 95 [PMID: 20015409 DOI: 10.1186/1471-230x-9-95]

77 **Malinen E**, Rinttilä T, Kajander K, Mättö J, Kassinen A, Krogius L, Saarela M, Korpela R, Palva A. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol* 2005; **100**: 373-382 [PMID: 15667495 DOI: 10.1111/j.1572-0241.2005.40312.x]

78 **Esmaili A**, Nazir SF, Borthakur A, Yu D, Turner JR, Saksena S, Singla A, Hecht GA, Alrefai WA, Gill RK. Enteropathogenic Escherichia coli infection inhibits intestinal serotonin transporter function and expression. *Gastroenterology* 2009; **137**: 2074-2083 [PMID: 19747920 DOI: 10.1053/j.gastro.2009.09.002]

79 **Nzakizwanayo J**, Dedi C, Standen G, Macfarlane WM, Patel BA, Jones BV. Escherichia coli Nissle 1917 enhances bioavailability of serotonin in gut tissues through modulation of synthesis and clearance. *Sci Rep* 2015; **5**: 17324 [PMID: 26616662 DOI: 10.1038/srep17324]

80 **Kekuda R**, Torres-Zamorano V, Leibach FH, Ganapathy V. Human serotonin transporter: regulation by the neuroprotective agent aurintricarboxylic acid and by epidermal growth factor. *J Neurochem* 1997; **68**: 1443-1450 [PMID: 9084414]

81 **Kubota N**, Kiuchi Y, Nemoto M, Oyamada H, Ohno M, Funahashi H, Shioda S, Oguchi K. Regulation of serotonin transporter gene expression in human glial cells by growth factors. *Eur J Pharmacol* 2001; **417**: 69-76 [PMID: 11301061]

82 **Gil C**, Najib A, Aguilera J. Serotonin transport is modulated differently by tetanus toxin and growth factors. *Neurochem Int* 2003; **42**: 535-542 [PMID: 12590935]

83 **Katsumata R**, Shiotani A, Murao T, Ishii M, Fujita M, Matsumoto H, Haruma K. Gender Differences in Serotonin Signaling in Patients with Diarrhea-predominant Irritable Bowel Syndrome. *Intern Med* 2017; **56**: 993-999 [PMID: 28458330 DOI: 10.2169/internalmedicine.56.7674]

84 **Galligan JJ**, Patel BA, Schneider SP, Wang H, Zhao H, Novotny M, Bian X, Kabeer R, Fried D, Swain GM. Visceral hypersensitivity in female but not in male serotonin transporter knockout rats. *Neurogastroenterol Motil* 2013; **25**: e373-e381 [PMID: 23594365 DOI: 10.1111/nmo.12133]

85 **Morales W**, Pimentel M, Hwang L, Kunkel D, Pokkunuri V, Basseri B, Low K, Wang H, Conklin JL, Chang C. Acute and chronic histological changes of the small bowel secondary to C. jejuni infection in a rat model for post-infectious IBS. *Dig Dis Sci* 2011; **56**: 2575-2584 [PMID: 21409374 DOI: 10.1007/s10620-011-1662-6]

86 **Shao YY**, Huang J, Ma YR, Han M, Ma K, Qin HY, Rao Z, Wu XA. Serum serotonin reduced the expression of hepatic transporter Mrp2 and P-gp via regulating nuclear receptor CAR in PI-IBS rats. *Can J Physiol Pharmacol* 2015; **93**: 633-639 [PMID: 26053941 DOI: 10.1139/cjpp-2015-0039]

87 **Ibeakanma C**, Ochoa-Cortes F, Miranda-Morales M, McDonald T, Spreadbury I, Cenac N, Cattaruzza F, Hurlbut D, Vanner S, Bunnett N, Vergnolle N, Vanner S. Brain-gut interactions increase peripheral nociceptive signaling in mice with postinfectious irritable bowel syndrome. *Gastroenterology* 2011; **141**: 2098-2108.e5 [PMID: 21856270 DOI: 10.1053/j.gastro.2011.08.006]

88 **Hsu LT**, Hung KY, Wu HW, Liu WW, She MP, Lee TC, Sun CH, Yu WH, Buret AG, Yu LC. Gut-derived cholecystokinin contributes to visceral hypersensitivity via nerve growth factor-dependent neurite outgrowth. *J Gastroenterol Hepatol* 2016; **31**: 1594-1603 [PMID: 26773283 DOI: 10.1111/jgh.13296]

89 **Sharma A**, Lelic D, Brock C, Paine P, Aziz Q. New technologies to investigate the brain-gut axis. *World J Gastroenterol* 2009; **15**: 182-191 [PMID: 19132768]

90 **Forsythe P**, Sudo N, Dinan T, Taylor VH, Bienenstock J. Mood and gut feelings. *Brain Behav Immun* 2010; **24**: 9-16 [PMID: 19481599 DOI: 10.1016/j.bbi.2009.05.058]

91 **Li H**, Cao Y. Lactic acid bacterial cell factories for gamma-aminobutyric acid. *Amino Acids* 2010; **39**: 1107-1116 [PMID: 20364279 DOI: 10.1007/s00726-010-0582-7]

92 **Bravo JA**, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, Bienenstock J, Cryan JF. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U S A* 2011; **108**: 16050-16055 [PMID: 21876150 DOI: 10.1073/pnas.1102999108]

93 **Zhao Z**, Nelson AR, Betsholtz C, Zlokovic BV. Establishment and Dysfunction of the Blood-Brain Barrier. *Cell* 2015; **163**: 1064-1078 [PMID: 26590417 DOI: 10.1016/j.cell.2015.10.067]

94 **Komarova Y**, Malik AB. Regulation of endothelial permeability via paracellular and transcellular transport pathways. *Annu Rev Physiol* 2010; **72**: 463-493 [PMID: 20148685 DOI: 10.1146/annurev-physiol-021909-135833]

95 **Zlokovic BV**. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat Rev Neurosci* 2011; **12**: 723-738 [PMID: 22048062 DOI: 10.1038/nrn3114]

**P-Reviewer:** Touil-Boukoffa C, Yu LCH **S-Editor:** Gong ZM

**L-Editor:** **E-Editor:**

**Specialty type:** Gastroenterology and hepatology

**Country of origin:** China

**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

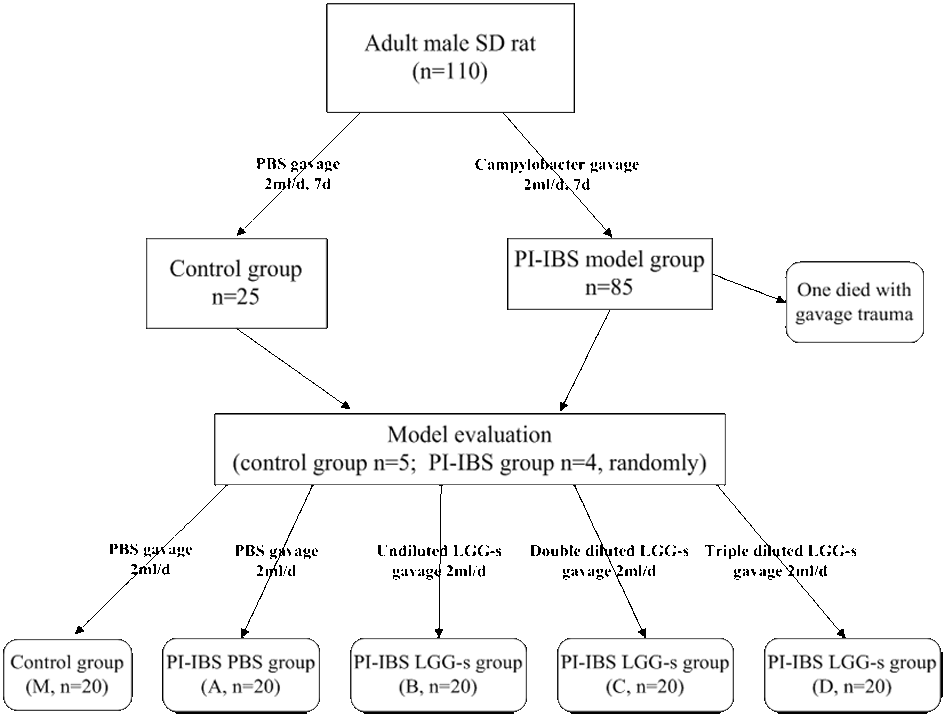
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Grade E (Poor): 0

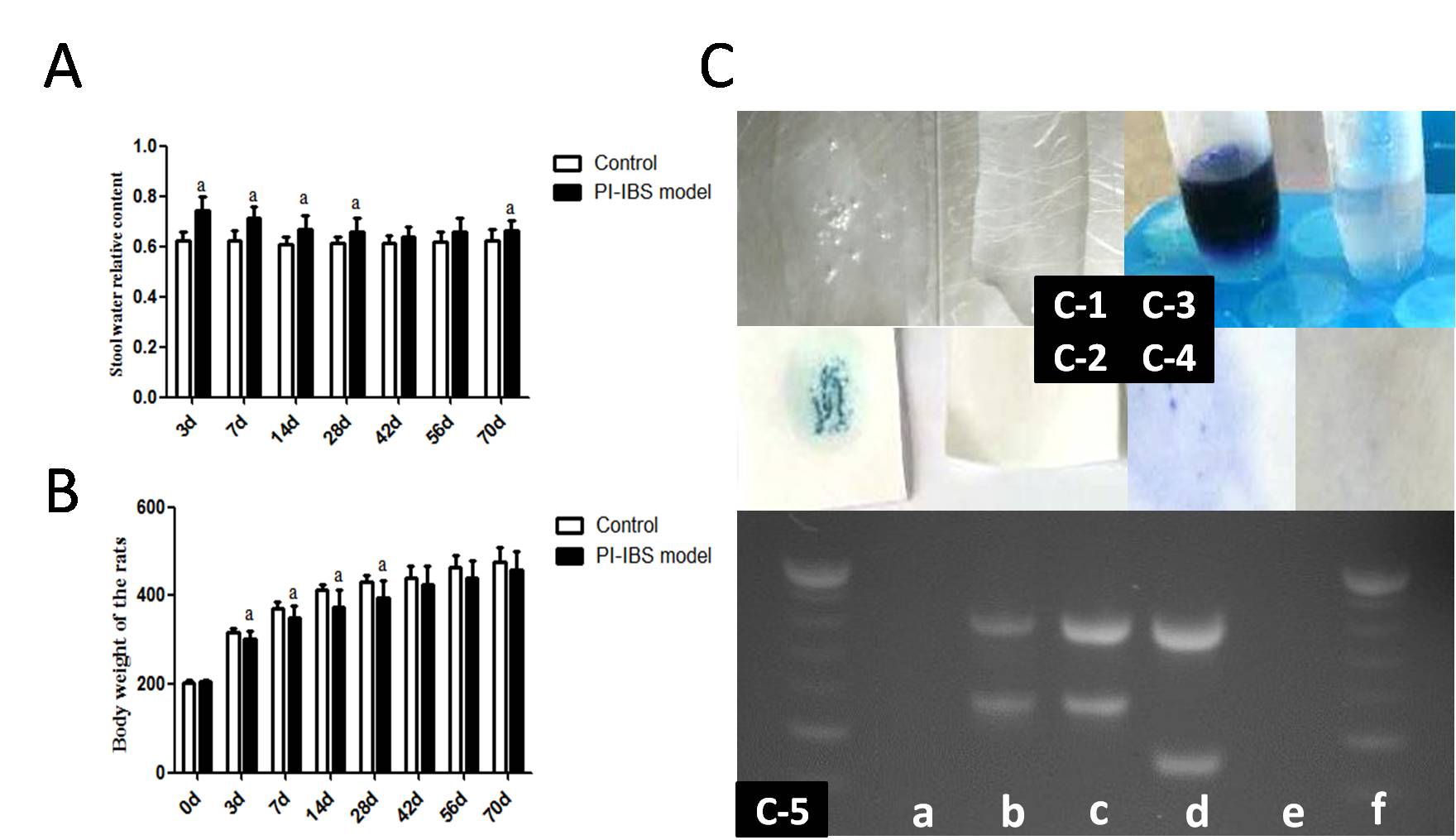
**Table 1 Primer sequences for RT-PCR**

|  |  |
| --- | --- |
| **Gene** | **Sequences (forward/reverse 5’-3’)** |
| *rGAPDH* | Sense: 5’-CCATCAACGACCCCTTCATT-3’ |
|  | Antisense: 5’-GACCAGCTTCCCATTCTCAG-3’ |
| *rSERT* | Sense: 5’-ACTGTTACCAAGATGCCCTG-3’ |
|  | Antisense: 5’-ATCTTCATTCCTCATCTCCGC-3’ |

rGAPDH: rat glyceraldehyde-3-phosphate dehydrogenase; rSERT: rat serotonin transporter.



**Figure 1 Experimental flow chart.**

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**Figure 2 Assessment of *Campylobacter* colonization phase.** A: Stool water relative content during the observation period, a*P* < 0.05 *vs* control group; B: Body weight of rats during the observation period, a*P* < 0.05 *vs* control group; C: C-1 catalase test, C-2 3-indoylacetate hydrolysis test, C-3 hippurate hydrolysis test, C-4 oxidase test, C-5 DNA agarose gel electrophoresis, a for negative simple, b for positive simple, c for positive quality control of Campylobacter jejuni, d for positive quality control of Campylobacter coli, e for negative quality control, f for DNA ladder. Control group *n* = 25; PI-IBS group *n* = 84.

**Figure 3 Assessment of PI-IBS phase.** A: Bristol Stool score of Bristol Stool score; B: Intestinal transit rate by activated carbon solution, ITR = length of the activated carbon moving in the bowel (cm)/length of the whole bowel (cm); C: AWR scores at different pressure. a*P* < 0.05 *vs* control group. Control group *n* = 5; PI-IBS group *n* = 4.

**Figure 4 Effects of LGG-s on SERT mRNA and SERT-P expression in rat intestinal tissues.** A: Form up to down represent the SERT mRNA level at the first, second, third and fourth week; B: Form up to down represents the SERT-P level at the first, second, third and fourth week analyzed by Western blot; C: Form up to down represents the quantitative analysis of SERT-P level at the first, second, third and fourth week analyzed by Western blot. b*P* < 0.05 *vs* B or C; c*P* < 0.05 *vs* all others; d*P* < 0.05 *vs* M or A; e*P* < 0.05 *vs* A; f*P* < 0.05 *vs* M, A or D; g*P* < 0.05 *vs* M, C or D; h*P* < 0.05 *vs* M or D. Control group *n* = 5; PI-IBS group *n* = 5, each week.



**Figure 5 Effects of LGG-s on SERT mRNA and SERT-P expression in rat brain tissues.** A: Form up to down represent the SERT mRNA level at the first, second, third and fourth week; B: Form up to down represents the SERT-P level at the first, second, third and fourth week analyzed by Western blot; C: Form up to down represents the quantitative analysis of SERT-P level at the first, second, third and fourth week analyzed by Western blot. Control group *n* = 5; PI-IBS group *n* = 5, each week.