Regulation of the serotonin transporter in the pathogenesis of irritable bowel syndrome

Duo-Chen Jin, Hai-Long Cao, Meng-Que Xu, Si-Nan Wang, Yu-Ming Wang, Fang Yan, Bang-Mao Wang

Duo-Chen Jin, Hai-Long Cao, Meng-Que Xu, Si-Nan Wang, Yu-Ming Wang, Fang Yan, Bang-Mao Wang, Department of Gastroenterology and Hepatology, General Hospital, Tianjin Medical University, Tianjin 300052, China

Hai-Long Cao, Fang Yan, Division of Gastroenterology, Hepatology and Nutrition, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN 372320696, United States

Author contributions: Jin DC, Cao HL and Wang BM designed the review; Jin DC, Cao HL, Xu MQ, Wang SN and Wang YM collected and analyzed the literature; Jin DC and Cao HL wrote the paper; Jin DC, Cao HL, Xu MQ, Wang SN, Yan F and Wang BM modified the manuscript; all authors were involved in the final approval of the article.

Supported by the National Natural Science Foundation of China, No. 81300272, No. 81470796, No. 81570489 and No. 81570478, and the Tianjin Research Program of Application Foundation and Advanced Technology of China, No. 15JCZDJC36600.

Conflict-of-interest statement: The authors have no conflicts of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Manuscript source: Invited manuscript

Correspondence to: Hai-Long Cao, MD, PhD, Department of Gastroenterology and Hepatology, General Hospital, Tianjin Medical University, 154 Anshan Road, Heping District, Tianjin 300052, China. cao_hailong@163.com
Telephone: +86-22-60362608
Fax: +86-22-27813550

Received: March 27, 2016
Peer-review started: March 28, 2016
First decision: May 12, 2016
Revised: May 28, 2016
Accepted: June 15, 2016
Article in press: June 15, 2016
Published online: September 28, 2016

Abstract

Serotonin (5-HT) and the serotonin transporter (SERT) have earned a tremendous amount of attention regarding the pathogenesis of irritable bowel syndrome (IBS). Considering that enteric 5-HT is responsible for the secretion, motility and perception of the bowel, the involvement of altered enteric 5-HT metabolism in the pathogenesis of IBS has been elucidated. Higher 5-HT availability is commonly associated with depressed SERT mRNA in patients with IBS compared with healthy controls. The expression difference of SERT between IBS patients and healthy controls might suggest that SERT plays an essential role in IBS pathogenesis, and SERT was expected to be a novel therapeutic target for IBS. Progress in this area has begun to illuminate the complex regulatory mechanisms of SERT in the etiology of IBS. In this article, current insights regarding the regulation of SERT in IBS are provided, including aspects of SERT gene polymorphisms, microRNAs, immunity and inflammation, gut microbiota, growth factors, among others. Potential SERT-directed therapies for IBS are also described. The potential regulators of SERT are of clinical importance and are important for better understanding IBS pathophysiology and therapeutic strategies.

Key words: Irritable bowel syndrome; Serotonin; Serotonin transporter; Regulation; Therapy

© The Author(s) 2016. Published by Baishideng Publishing Group Inc. All rights reserved.
Core tip: The serotonin transporter (SERT) participates in metabolizing serotonin in the gut and plays a crucial role in the pathogenesis of irritable bowel syndrome (IBS). This review summarizes the relevant evidence on the factors that might regulate SERT, including SERT gene polymorphisms, microRNAs, immunity and inflammation, gut microbiota and growth factors. This review also reveals several potential treatments targeting SERT for IBS patients.

INTRODUCTION

As a functional bowel disorder, irritable bowel syndrome (IBS) has the highest incidence rate worldwide. IBS is defined as a disorder with complex symptoms appearing as abdominal pain/discomfort and altered bowel patterns[1-3]. A growing number of people suffer from IBS, with an estimated 5.8%-17.5% prevalence, especially in females[4,5]. IBS causes a tremendous decline in the health-related quality of life and brings a considerable socioeconomic burden of up to $19 billion[2,6]. The Rome III criteria have been improved to help with the diagnosis and differential diagnosis of the syndrome[7-10]. According to these criteria, 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 unsubtyped (IBS-U)[11,12]. Furthermore, a 6-year follow-up study showed that approximately 10% of patients with infective gastroenteritis suffer from post-infective IBS (PI-IBS)[13]. Because IBS is considered to be a multifactorial and heterogeneous disease with various phenotypes, no single mechanism entirely explains the pathophysiology of the disorder. Some possible mechanisms involve the initiation, persistence and severity of symptom flares, including inflammation, immunity, infection[14-16], the gut microbiota[16,17], psychosocial stress[16,18,19] and an abnormal brain-gut axis[16,20]. Recent discoveries have revealed that genetic susceptibility[21], diet/drug intolerances[20] and environmental pollutants[21] are closely associated with IBS pathogenesis. Although the etiology of IBS is largely elusive, there are some characteristic symptoms of the disorder, including visceral hypersensitivity[16,24], intestinal barrier dysfunction[25] and gut motility disorder[16,17,20].

As a signal transducer and a neurotransmitter, serotonin (5-HT) mediates intercellular signaling transmission in the gut, and most of the 5-HT in the body is in the gut. Enteric 5-HT is synthesized by enterochromaffin (EC) cells (90%) and enteric serotonergic neurons of the myenteric plexus (10%)[27]. Therefore, EC cells are the main source of enteric 5-HT in the gastrointestinal (GI) tract[28,29]. 5-HT inactivation is as important as 5-HT release for maintaining dynamic equilibrium. As a number of neurotransmitter sodium symporters or the solute carrier superfamily 6, the serotonin reuptake transporter (SERT) plays an irreplaceable role in 5-HT inactivation by removing 5-HT from the interstitial space in the lamina propria into mucosal enterocytes and presynaptic neurons that are responsible for catabolism[30,31]. Coates et al[31] first characterized a significantly decreased level of SERT in IBS. However, there was another conflicting finding of increased SERT expression in IBS[32,33]. Taking the significant differences in the analytical methodology used and the heterogeneity of phenotypes into account, most researchers, such as Faure et al[34], have demonstrated that IBS patients have a remarkably attenuated level of SERT expression in the intestinal lining, which conforms to a remarkably decreased capacity of enterocytes to reuptake 5-HT. It is generally accepted that there is a significant inverse correlation targeting the level of availability between SERT and 5-HT.

SERT plays a critical role in the uptake and internalization of extracellular 5-HT. Previous studies have provided support to the concept that SERT is regulated by transcriptional and posttranslational mechanisms. To date, an association between SERT gene polymorphisms and IBS susceptibility has been inconsistent among different ethnic groups and even among different populations[35]. Despite the lack of consensus on the wide range of roles of potential factors, immunity activation, inflammatory response, gut microbiota and their relationships have been suggested to regulate SERT expression in PI-IBS[36]. Probiotics are also notable for linking inflammation-immune systems and gut microbiota in IBS patients[37]. Recent studies have also shed light on the fascinating roles of microRNAs, growth factors and other factors in regulating SERT[38].

ROLE OF SERT IN IBS

5-HT expands its regulatory functions outside the central nervous system as a neurotransmitter. In the gut, 5-HT is also a key signal transducer[39,40]. Although the complex roles of 5-HT in the gut have not yet been clearly and completely elucidated, current studies have proven that 5-HT acts upon mucosal sensory transduction, responding to pressure and luminal stimuli derived from diet and bacteria[41]. The release of 5-HT acting on a series of 5-HT receptors initiates secretory reflexes, peristaltic reflexes and, if pronounced, diarrhea, by stimulating intrinsic primary afferent neurons and myenteric interneurons[41-43]. Furthermore, by stimulating extrinsic sensory nerves, 5-HT can also transmit the sensation of discomfort to
the central nervous system along the gut–brain axis in IBS. Therefore, 5-HT is closely related to secretion, motility and sensation in the gut. Shufflebotham et al.\(^{[44]}\) highlighted the importance of 5-HT dysfunction in IBS symptoms and psychophysiological manifestation with the use of the acute tryptophan depletion paradigm. Moreover, increasing evidence suggests that psychiatric comorbidities are highly prevalent in IBS patients\(^{[45]}\). Antidepressant selective serotonin reuptake inhibitors (SSRIs) are considered to be possible treatments for IBS. In 2014, a systematic review declared that antidepressants are effective in treating IBS\(^{[46]}\). However, in 2015, a meta-analysis with conflicting results found that the efficacy of SSRIs to treat IBS was inconclusive\(^{[47]}\). One study showed that IBS patients with a psychiatric comorbidity had a greater probability of carrying SERT variants\(^{[48]}\). The possibilities underpinning antidepressants, such as SSRIs and other factors that regulate SERT, require further elaboration.

Termination of the 5-HT signal is as important as its initiation; therefore, SERTs on the cell membrane of enterocytes are vital to transport 5-HT intracellularly, where 5-HT is metabolized by monoamine-oxidases\(^{[49]}\). Using mice with a targeted deletion of SERT, Chen et al.\(^{[50]}\) demonstrated that nearly all of the intestinal epithelial cells on the surface of the lumen express SERT. As a result, it is not surprising that the intestinal mucosa has a huge capacity for taking up 5-HT from the intestinal space. Therefore, 5-HT is transported into enterocytes by SERT after release from EC cells and acting on local selected receptors\(^{[50]}\). As a membrane-embedded transporter, SERT is crucial for modulating the amplitude and duration of the 5-HT signal\(^{[51]}\). As discussed previously, a significant correlation has been observed between abnormalities of 5-HT signaling and IBS-like pathogenesis. Furthermore, it is now believed that altered SERT expression is responsible for disorganized 5-HT signaling. When dysregulated SERT increases mucosal 5-HT availability, high-levels of gut secretion and motility might accelerate the development of IBS-D\(^{[52]}\). It is generally accepted that the abnormalities of SERT expression contribute to IBS development. However, the regulation of SERT expression in IBS and the underlying mechanisms are not fully understood.

### POTENTIAL REGULATORY FACTORS OF SERT

Both genetic and non-genetic factors are implicated in the up-regulation or down-regulation of SERT expression in IBS (Table 1). It is becoming clear that genetic predisposition might underlie IBS in individuals\(^{[53]}\). A large-scale study between monozygotic twins and dizygotic twins proved that both heredity and the environment contribute to the development of IBS. Furthermore, it appeared that environmental influence was more important for individuals than heredity in IBS\(^{[54]}\). In the present article, the potential regulatory factors of SERT expression are presented and discussed, and these factors might be involved in the pathophysiology and/or etiology of IBS.

#### SERT gene polymorphisms

As Hotoleanu et al.\(^{[55]}\) demonstrated using twin studies, familial aggregation and epidemiology, genetic factors contribute to IBS, especially polymorphisms of the SERT gene. In other words, a low-expression SERT genotype might underlie a genetic predisposition to IBS\(^{[56,57]}\). Furthermore, Kohen et al.\(^{[58]}\) reported a trend

---

### Table 1 Summary of potential regulators of the serotonin transporter in irritable bowel syndrome

| Regulatory factors | Ref. | Publication year | Study type |
|--------------------|------|-----------------|------------|
| SERT polymorphisms |      |                 |            |
| 5-HTTLPR           | Zhang et al.\(^{[59]}\) | 2014 | Meta-analysis |
|                    | Areeshi et al.\(^{[60]}\) | 2013 | Meta-analysis |
|                    | Wang et al.\(^{[61]}\) | 2012 | Case-control study |
|                    | Yeo et al.\(^{[62]}\) | 2004 | Case-control study |
|                    | Kumar et al.\(^{[63]}\) | 2012 | Case-control study |
|                    | Sikander et al.\(^{[64]}\) | 2009 | Case-control study |
|                    | Pata et al.\(^{[65]}\) | 2002 | Case-control study |
| Stin2 VNTRs         | Wang et al.\(^{[66]}\) | 2004 | Case-control study |
|                    | Yeo et al.\(^{[67]}\) | 2004 | Case-control study |
| SNPs               | Kohlen et al.\(^{[68]}\) | 2009 | Case-control study |
| MicroRNAs (miR)    |       |                 |            |
| MiR-16             | Baudry et al.\(^{[69]}\) | 2010 | Experimental study |
| MiR-545            | Jensen et al.\(^{[70]}\) | 2009 | Experimental study |
| MiR-15a            | Moya et al.\(^{[71]}\) | 2013 | Experimental study |
| MiR-24             | Liao et al.\(^{[72]}\) | 2016 | Case-control study |
| Immunity and inflammation |       |                 |            |
| Immune cells       |       |                 |            |
| IELs               | Foley et al.\(^{[73]}\) | 2011 | Experimental study |
|                    | Faure et al.\(^{[74]}\) | 2010 | Experimental study |
| Mast cells         | Foley et al.\(^{[75]}\) | 2011 | Experimental study |
| T cells            | Wheatcroft\(^{et al.}[76]\) | 2005 | Experimental study |
|                    | Faure et al.\(^{[77]}\) | 2010 | Experimental study |
| Inflammatory cytokines |       |                 |            |
| IFN-γ and TNF-α    | Foley et al.\(^{[78]}\) | 2007 | Experimental study |
| TGF-β (T)          | Nazir et al.\(^{[79]}\) | 2015 | Experimental study |
| Gut microbiota     |       |                 |            |
| EPEC (E. coli)     | Esmaili et al.\(^{[80]}\) | 2009 | Experimental study |
| EcN (N. Enterica)  | Nizakirwanayo et al.\(^{[81]}\) | 2015 | Experimental study |
| LGG (L. Casei)     | Wang et al.\(^{[82]}\) | 2015 | Experimental study |
| Growth factors (f) |       |                 |            |
| EGF                | Kekuda et al.\(^{[83]}\) | 1997 | Experimental study |
| bFGF               | Kubota et al.\(^{[84]}\) | 2001 | Experimental study |
| NGF                | Gil et al.\(^{[85]}\) | 2003 | Experimental study |

5-HTTLPR: 5-HT-transporter-gene-linked polymorphic region; Stin2 VNTRs: Variable number of tandem repeats Stin2; SNPs: Single nucleotide polymorphisms; IELs: Intestinal epithelial lymphocytes; IFN-γ and TNF-α: Interferon-γ and tumor necrosis factor-α; TGF-β1: Transforming growth factor-β1; EPEC: Enteropathogenic E. coli; EcN: Escherichia coli Nissle 1917; LGG: Lactobacillus rhamnosus GG supernatant; EGF: Epidermal growth factor; bFGF: Basic fibroblast growth factor; NGF: Nerve growth factor.
towards an association between 5-HT-transporter-gene-linked polymorphic region (5-HTTLPR) L/L genotype and IBS. However, Camilleri et al.[60] found that colonic mucosal expression of the SERT gene was normal in IBS. Galligan et al.[60] found increased serotonin availability in SERT knockout rats associated with visceral hypersensitivity. The SERT gene, solute carrier family 6 member 4 (SLC6A4), localizes to chromosome 17q12. SLC6A4 spans approximately 40 KB, contains 14 exons and ultimately encodes a 603-amino acid protein.[61-63] There are a series of polymorphic regions that might affect the expression or function of SERT[59,64-67] and further alter 5-HT reuptake, reaching up to 40-fold in vitro.[68] Current research mainly focuses on positive associations of the SLC6A4 genetic polymorphisms with the etiology of IBS, including 5-HTTLPR,[69] a variable number of tandem repeats (VNTR) STin2,[65] and functional single nucleotide polymorphisms (SNPs; rs25531 and rs25532, etc.)[58,70,71]. However, the presence of linkage disequilibrium between the three aspects has not yet been determined.[58]

The most frequently studied variant, a 5-HTTLPR insertion/deletion polymorphism of approximately 44 base pairs, is subdivided into long (L) and short (S) alleles.[69,72]. Furthermore, compared with the L/S and S/S genotypes, the transcriptional efficiency of the L/L genotype is significantly higher.[73] Our previous study found that the L/L genotype leading to a higher SERT level appeared more frequently in IBS-C individuals than in IBS-D and healthy individuals.[73] Yeo et al.[74] reported that the 5-HTTLPR polymorphism was highly related to female patients with IBS. The S allele leading to decreased transcription of SLC6A4 and attenuated expression of SERT protein resulted in a reduced reuptake of 5-HT and a higher S-HT level, which was consistent with manifestations of IBS-C compared with other subtypes of IBS and controls.[75] Contradictorily, Sikander et al.[76] and Pata et al.[77] reported that the S/S genotype had a significant correlation with IBS-C patients in the Indian and Turkish population, and Wendelbo et al.[33] concluded an increased content of SERT availability in ileal epithelia facilitating the pathogenesis of IBS, regardless of the subtype. However, because of insufficient patients participating in these studies, there was still no consistent conclusion. A meta-analysis containing thousands of IBS cases found ethnic differences in the relationship between 5-HTTLPR and IBS; moreover, the L/L genotype, or rather the L allele, was more relevant to IBS-C in East Asians than in Caucasians.[78]

Similarly, another meta-analysis showed that the SLC6A4 polymorphism is associated with a reduced risk of IBS in American and Asian populations.[65] Another SERT gene polymorphism, called variable number of tandem repeats STin2, or simply “STin2 VNTR” for short, is located in intron 2 and consists of an indeterminate number of 17-bp segments (i.e., 9, 10 or 12 repeats).[65,70] Our previous study reported that the 10/12 genotype might contribute to IBS[79], although other reports regarding the association between STin2 VNTRs and IBS were controversial and inconclusive.[74,80] With regard to functional SNPs within the VNTR promoter, Kohen et al.[80] found that compared with the more frequent A-allele, the comparatively rare rs25531 G-allele decreased SERT transcription and thus increased the IBS risk by approximately 3-fold. SERT gene promoter polymorphisms have been implicated in the treatment effects of histone deacetylase inhibitors (butyrate or trichostatin) in cultured colonic epithelial cells (Caco-2 cells), which resulted in reduced SERT mRNA and protein expression by suppressing the human SERT (hSERT) promoter 1.[81] The development of SERT gene-specific therapeutics to regulate SERT expression in the treatment of multiple disorders, including IBS, is realizable. Clinicians could put individualized treatment into effect according to different SERT genotypes as one of the factors.

MicroRNAs
Posttranscriptional gene regulation by microRNAs (miRNAs) can greatly contribute to miRNA-targeted gene translation.[62,83] miRNAs, endogenous about 22 nucleotide (nt) noncoding RNAs, pair with and then silence target mRNAs and achieve fine adjustments of protein outputs.[64,66]. Of interest, nearly all aspects of biological processes, including development and cellular homeostasis, are under the influence of miRNAs. Moreover, miRNAs can facilitate the development of several types of diseases when they dysregulate targeted gene expression.[83-85,87] Despite insufficient studies focusing on the 3’-untranslated region (3’-UTR) of SLC6A4, miRNA binding to the 3’-UTR of SERT mRNAs by incomplete complementary base pairing is crucial for SERT mRNA translation, localization and stability.[83-86]. Despite insufficient studies focusing on the 3’-untranslated region (3’-UTR) of SLC6A4, miRNA binding to the 3’-UTR of SERT mRNAs by incomplete complementary base pairing is crucial for SERT mRNA translation, localization and stability.[83-86].

During the past several years, it has been shown that SERT is a target of microRNA-16 (miR-16). The highly conserved miR-16 among mammalian species has high expression levels in the heart, brain, small intestine, lung and kidney.[89,90] Baudry et al.[80] investigated if SERT expression was decreased by miRNAs in monoaminergic neurons utilizing the 1C11 neuroectodermal cell line expressing SERT transcripts. The results showed a 40% decline in the numbers of [3H]-paroxetine (SSRI) binding sites after transfection with a high level of miR-16. SSRI fluoxetine down-regulated SERT expression by increasing the level of miR-16 in 1C11 cells (1C11 neuroectodermal cells differentiate into serotonergic neuronal cells).[80] Similar findings were obtained in the hippocampus, showing that fluoxetine treatment resulted in down-regulated miR-16 and 5-fold increased SERT expression, with further illustration that the level of miR-16 was regulated by SSRI antidepressants and was increased or decreased according to the different regions in
the brain. Furthermore, the neutralization of miR-16 played an antidepressant role in the hippocampus. Direct injection of anti-miR-16 had an antidepressant effect similar to fluoxetine. A study investigating acute lung injury also drew the same conclusions that decreased miR-16 levels contributed to increased SERT expression and therefore promoted the pathogenesis of pulmonary edema.

miR-16 might not be the only modulatory miRNA involved in the translational repression of SERT. For example, Jensen and colleagues found that SERT expression in the HeLa cell line was also regulated by miR-545, and a U to G SNP in the 3’-UTR of the SERT mRNA had no effect on miR-545 binding and SERT down-regulation. In addition, miR-15a contiguously located at chromosome 13q14.3 with miR-16 also regulated SERT expression in rat and human cells. More concerning, the observed results from the brain tissue of Wistar rat pups highlighted that Cronobacter sakazakii infection up-regulated miR-16 expression interacting with SERT mRNA, which led to decreased levels of 5-HT and SERT expression. Recently, a study directly illuminated that increased miR-24 expression in the enterocytes of IBS patients and mouse models promoted IBS-D pathogenesis by down-regulating SERT expression. Discovering novel miRNAs related to posttranscriptional SERT gene regulation and elucidating the underlying mechanisms provide a new strategy to expand our understanding of miRNAs in the development and treatment of IBS.

**Immunity and inflammation**

Given that accumulating evidence points to a critical role for immune activation of the gut mucosa in EC cell hyperplasia and reduced SERT activity in IBS-D patients or post-infectious IBS (PI-IBS) patients, it is not surprising that mucosal 5-HT is increased in IBS-D patients and PI-IBS patients. It is generally accepted that there are increased levels of mucosal immune cell infiltration and proinflammatory cytokines in IBS patients. Furthermore, the inflammatory state of the intestinal mucosa promotes visceral hypersensitivity. Evidence suggests that 50% of IBS patients exhibit a drastic 72% increase of immunocytes in colonic mucosa, including CD3+, CD4+ and CD8+ T cells and mast cells, compared with healthy controls. Foley et al. found that the reduced level of mucosal SERT mRNA in IBS-D patients was correlated with increased numbers of mucosal intraepithelial lymphocytes (IELs) and mast cells compared with healthy controls. A study from Wheatcroft and colleagues evaluated post-Trichinella spiralis infection of T cell receptor (TCR) knockout mice with respect to EC cell numbers and SERT expression. The authors demonstrated that deficiencies of all T cells decreased infection-induced EC cell hyperplasia and extinguished mastocytosis, with a drastic reduction in jejunal SERT expression. Paradoxically, despite the general presence of inflammatory infiltrates, Faure et al. detected no differences in the numbers of IELs and CD3+ cells located in the lamina propria between IBS patients and healthy controls.

Accumulating evidence has demonstrated that proinflammatory mediators, such as interferon-γ and tumor necrosis factor (TNF-α), and not solely a non-specific change of inflammatory damage on epithelial cells, induce significant reductions in SERT mRNA, SERT protein levels and SERT function in Caco2 cells. However, prostaglandin E and interleukin-12 (IL-12) had no effect on the SERT mRNA and protein levels. Furthermore, treatment with Shugan decoction, a type of traditional Chinese medicine used to treat IBS-D patients, resulted in a decreased TNF-α level with up-regulated SERT gene and protein levels in colonic tissue, which suggested underlying interactions between TNF-α and SERT expression. A protective cytokine, transforming growth factor-β1, can activate SERT activity and inhibit intestinal inflammation via PI3K and syntaxin 3. These studies provide an overview of immune mechanisms involved in SERT regulation in a subset of IBS patients.

**Gut microbiota**

It is generally accepted that gut microbiota dysbiosis is responsible for intestinal ecology disturbances, which could be a significant catalyst in the development of functional bowel disorders. The current insight is that gut host-microbial interactions are important elements involved in the pathogenesis of IBS because of the convincing findings that predisposed individuals following infectious gastroenteritis suffer from PI-IBS and resemble patients with IBS-D. Because of the rapid evolution of analytical techniques, such as 16S rRNA-based microbiota analyses for profiling bacteria in the GI tract, not just in culture, it has been shown that mucosal and fecal gut microbial community composition differs between patients with IBS and healthy controls. Albeit with significant differences in methods, many studies have found that the relative abundances of the genera Lactobacillus, Bifidobacterium, Actinobacteria and Bacteroidetes were decreased, while Proteobacteria, Firmicutes and Firmicutes: Bacteroidetes ratios were increased in fecal samples of IBS-D patients. Malinen et al. even found an association between altered bacterial composition and subtypes of IBS, with a decreased amount of Lactobacillus spp. among IBS-D patients and an elevated amount of Veillonella spp. among IBS-C patients. However, the lack of large sample sizes and the heterogeneity of IBS symptoms represent limitations of these studies.

As noted previously, particular gut microbes and microbial metabolites regulate tryptophan metabolism, the serotonergic system and brain-gut axis functions and thereby alter the levels of 5-HT in the colon and blood, which might suggest a critical role for...
the intestinal flora in regulating SERT and ultimately influencing the pathogenesis of IBS. Yano et al. found that EC cells were promoted to synthesize and secrete 5-HT by endogenous bacteria, such as spore-forming bacteria and their metabolites in germ-free mice. Esmaili et al. found that Caco-2 cells and mice infected by enteropathogenic E. coli to simulate infectious diarrheal diseases (PI-IBS and enteric infections) had decreased SERT mRNA levels, apical SERT activity, 5-HT uptake and mucosal 5-HT content. An investigation by Nizakizwanyo et al. demonstrated that the exposure of mouse ileal tissue to E. coli Nissle 1917 in vitro increased 5-HT bioavailability and decreased its metabolite level [5-hydroxy indole acetic acid (5-HIAA)], which suggested the underlying mechanisms for clearing 5-HT by SERT. Similarly, in IBS, reduced 5-HIAA levels and 5-HIAA/5-HT ratios elucidate serotonergic system dysbiosis with regard to both synthesis and metabolism. Our previous study suggested that the supernatant of probiotics, such as Lactobacillus rhamnosus GG, up-regulated the SERT mRNA level as much as 9.4-fold in enterocytes and mouse intestinal tissues in a concentration- and time-dependent manner. Our research also found that a protein derived from LGG, known as p40, activated epidermal growth factor receptor (EGFR), which suggested that LGG up-regulated SERT possibly by activating EGFR.

Therapeutic strategies targeting the gut microbiota to recover the decreased diversity and stability might be a viable treatment strategy for IBS and other 5-HT-related brain-gut-microbiota axis disorders. To date, scientists and clinicians have made a variety of creative attempts, especially using probiotics, prebiotics, antibiotics and fecal microbiota transplantation (FMT), to increase the relative abundance of commensals (such as Lactobacilli and Bifidobacteria, etc.) and conversely, to decrease the relative abundance of those bacterial species exacerbating IBS symptoms (Clostridium, E. coli, Salmonella, Shigella and Pseudomonas). Both a low-carbohydrate diet and the probiotic LGG have been proven effective in IBS patients. Lactococcus lactis, which is effective in suppressing colon inflammation by secreting IL-10, restores colonic 5-HT concentrations, given that the 5-HT level is increased in a dinitro-benzenesulfonic-acid micro-inflammation model. Martin et al. found that the probiotic Faecalibacterium prausnitzii strain A2-165 (a type of commensal bacterium) or its supernatant had anti-inflammatory effects, with down-regulation of 5-HT levels to restore the normal state. Rifaximin, the most studied antibiotic in IBS, increased the relative abundance of Lactobacillus in the ileum, which relieved the mucosal inflammatory state and visceral hyperalgesia of the rat model. There is growing evidence regarding the efficacy of FMT in relieving symptoms in IBS patients, even in patients with longstanding refractory IBS-D, via restoring the intestinal microbiota. However, no study has demonstrated a relationship between FMT and SERT in IBS. Further studies are necessary to determine new classes of probiotics and underlying mechanisms contributing to the treatment of IBS; meanwhile, the feasibility and reliability of FMT remain to be determined.

**Growth factors**

There is growing evidence regarding the role of growth factors, such as EGF, basic fibroblast growth factor and nerve growth factor, in the up-regulation of SERT expression. At present, EGF has been the most studied of these factors. As a polypeptide with 53 amino acid residues and growth hormone, EGF plays multiple biological roles by combining with a specific EGFR located on the basolateral surface of enterocytes. There is evidence to suggest that EGF is involved in many normal physiological processes (stimulating intestinal epithelium cell proliferation, differentiation and maturation, etc.) and pathophysiologic situations (maintenance of homeostasis, protection and regeneration of gastrointestinal mucosa). Given that EGF signaling protects the GI tract from intestinal inflammation, little is known about a potential correlation between EGF signaling and IBS pathogenesis.

In response to SERT regulation, as Gill et al. first suggested, EGF acting on EGFR activates the hSERT promoter and upregulates SERT mRNA levels and function in enterocytes through transcriptional mechanisms in a dose- and time-dependent manner. Two types of alternate promoters of the SERT gene, hSERTp1 and hSERTp2, are both active in Caco-2 cells by approximately 2- to 2.5-fold, respectively, compared with the transfected results of the pGL2 empty vector alone. Accumulating evidence suggests that EGF promotes SERT gene expression. Kekuda et al. found that the treatment of human placental choriocarcinoma cells with EGF increased the levels of SERT transcriptional activity, SERT mRNA expression and SERT function, likely by activating the EGFR receptor through tyrosine phosphorylation. Kubota et al. reached similar conclusions about EGF and basic fibroblast growth factor using human glial cells (astrocytes). However, the positive effects of EGF on both distinct promoters of the SERT gene (hSERTp1 and hSERTp2) are counteracted by inhibiting EGFR tyrosine kinase activity. Decreased plasma and colonic tissue EGF levels were observed in IBS patients and in a rat model with visceral hypersensitivity. Therefore, decreased EGF correlates with decreased SERT activity, which is consistent with the conclusions that decreased EGF levels result in decreased removal of 5-HT into intestinal epithelial cells, stimulating visceral sensitivity and ultimately contributing to IBS.
suggest that the up-regulation of SERT expression and function by growth factors might provide a better understanding of the pathogenesis and treatment of IBS.

Others
In addition, several different factors modulate SERT expression. As an agonist of tyrosine-kinase receptors, aurantricarboxylic acid plays a role in the upregulation of SERT, similar to EGF. Although studies have found that some factors (CCAAT/enhancer binding protein beta, heterogeneous nuclear ribonucleoprotein K, 10(-7)M 4-beta-12-tetradecanoylphorbol-13-acetate, etc.) regulate SERT, it remains to be determined if these factors are involved in IBS pathogenesis.

FUTURE PROSPECTS
It is now believed that 5-HT signaling is essential to the pathogenesis of IBS. As a result, new therapeutic strategies targeting the abnormal expression of SERT might represent a breakthrough to relieve the symptoms of this excruciating disease. At present, therapeutic approaches targeting gut microbiota, immune activation and the inflammatory response have received adequate attention to regulate SERT. There is no doubt that these potential regulators of SERT hold great promise for the development of treatments for IBS.

REFERENCES
1 Jarrett ME, Han CJ, Cain KC, Burr RL, Shulman RJ, Barney PG, Naliboff BD, Zia J, Hertikemper MM. Relationships of abdominal pain, reports to visceral and temperature pain sensitivity, conditioned pain modulation, and heart rate variability in irritable bowel syndrome. *Neurogastroenterol Motil* 2016; 28: 1094-1103 [PMID: 26993039 DOI: 10.1111/nmo.12812]
2 Sayuk GS, Gyawali CP. Irritable bowel syndrome: modern concepts and management options. *Am J Med* 2015; 128: 817-827 [PMID: 25731138 DOI: 10.1016/j.amjmed.2015.01.036]
3 Guagnazzi D, Arias A, Lucendo AJ. Systematic review with meta-analysis: diagnostic overlap of microscopic colitis and functional bowel disorders. *Aliment Pharmacol Ther* 2016; Epub ahead of print [PMID: 26913568 DOI: 10.1111/apt.13573]
4 Kaji M, Fujitani W, Shiba M, Kohata Y, Yamagami H, Tanigawa T, Watanabe K, Watanabe T, Tominaga K, Arakawa T. Prevalence of irritable bowel syndrome in secondary care. *World J Gastroenterol* 2014; 20: 127 [PMID: 22783191 DOI: 10.3389/fphar.2012.00127]
5 Sperber AD, Dumitrascu D, Fukudo S, Gershon C, Ghoshal UC, Gwee KA, Hungin AP, Kang YJ, Minhu C, Schmulson M, Bolotin A, Friger M, Freda T, Whitehead W. The global prevalence of IBS in adults remains elusive due to the heterogeneity of studies: a Rome Foundation working team literature review. *Gut* 2016; Epub ahead of print [PMID: 26818616 DOI: 10.1136/gutjnl-2015-311120]
6 Wilson A, Longstreth GF, Knight K, Wong J, Wade S, Chiou CF, Barghout V, Frech F, Ofman JJ. Quality of life in managed care patients with irritable bowel syndrome. *Manag Care Interface* 2004; 17: 24-28, 34 [PMID: 15038690]
7 Ford AC, Berck P, Morgan DG, Bolino C, Pinto-Sanchez MI, Moayyedi P. Validation of the Rome III criteria for the diagnosis of irritable bowel syndrome in secondary care. *Gastroenterology* 2013; 145: 1262-1270.e1 [PMID: 23994201 DOI: 10.1053/j.gastro.2013.08.048]
8 Engsbro AL, Beigrad LM, Kjeldsen J, Larsen PV, de Mackaudel OS, Jarbol DE, Bytzer P. Patients suspected of irritable bowel syndrome–cross-sectional study exploring the sensitivity of Rome III criteria in primary care. *Am J Gastroenterol* 2013; 108: 972-980 [PMID: 23419383 DOI: 10.1038/ajg.2013.15]
9 Wang X, Luscombe GM, Boyd C, Kellow J, Abraham S. Functional gastrointestinal disorders in eating disorder patients: altered diagnosis and predictors using ROME III compared to ROME II criteria. *World J Gastroenterol* 2014; 20: 16293-16299 [PMID: 25473186 DOI: 10.3748/wjg.v20.i43.16293]
10 Koloski NA, Jones M, Young M, Talley NJ. Differentiation of functional constipation and constipation predominant irritable bowel syndrome based on Rome III criteria: a population-based study. *Aliment Pharmacol Ther* 2015; 41: 856-866 [PMID: 25736433 DOI: 10.1111/ajp.13149]
11 Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. *Gastroenterology* 2006; 130: 1480-1491 [PMID: 16675861 DOI: 10.1016/j.gastro.2005.11.061]
12 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]
13 Neal KR, Barker L, Spiller RC. Prognosis in post-infective irritable bowel syndrome: a six year follow up study. *Gut* 2002; 51: 410-413 [PMID: 12171965]
14 O’Malley D. Immuno modulation of enteric neural function in irritable bowel syndrome. *World J Gastroenterol* 2015; 21: 7362-7366 [PMID: 26139983 DOI: 10.3748/wjg.v21.i24.7362]
15 Ohman L, Simren M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nat Rev Gastroenterol Hepatol* 2010; 7: 163-173 [PMID: 20101257 DOI: 10.1038/nrgastro.2010.4]
16 Moloney RD, Johnson AC, O’Malony SM, Dinan TG, Greenwood-Van Meerveld B, Cryan JF. Stress and the Microbiota-Gut-Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome. *Front Cell Neurosci* 2016; 10: 127 [PMID: 26662472 DOI: 10.3389/fncel.2016.00127]
17 Ringel-Kulka T, Choi CH, Temas D, Kim A, Maier DM, Scott K, Galanko JA, Ringel Y. Altered Colonic Bacterial Fermentation as a Potential Pathophysiological Factor in Irritable Bowel Syndrome. *Am J Gastroenterol* 2015; 110: 1339-1346 [PMID: 26303129 DOI: 10.1038/ajg.2015.220]
18 Mawe GM, Coates MD, Moses PL. Review article: intestinal serotonin signalling in irritable bowel syndrome. *Aliment Pharmacol Ther* 2006; 23: 1067-1076 [PMID: 16611266 DOI: 10.1111/j.1365-2036.2006.02838.x]
19 Qin HY, Cheng CW, Tang XD, Bian ZX. Impact of psychological stress on irritable bowel syndrome. *World J Gastroenterol* 2014; 20: 14126-14131 [PMID: 25339801 DOI: 10.3748/wjg.v20.i39.14126]
20 Fichna J, Storr MA. Brain-Gut Interactions in IBS. *Front Pharmacol* 2012; 3: 127 [PMID: 22783191 DOI: 10.3389/fphar.2012.00127]
21 Gazouli M, Wouters MM, Kapur-Pojskic L, Bengtson MB, Friedman E, Nikčević G, Demetriou CA, Mulak A, Santos J, Niesler B. Lessons learned—resolving the enigma of genetic factors in IBS. *Nat Rev Gastroenterol Hepatol* 2016; 13: 77-87 [PMID: 27626033 DOI: 10.1038/nrgastro.2015.206]
22 Gibson PR, Varney J, Malakar S, Muir JG. Food components and irritable bowel syndrome. *Gastroenterology* 2015; 148: 1158-74.e4 [PMID: 25680668 DOI: 10.1053/j.gastro.2015.02.005]
23 Marynowski M, Likońska A, Zatorski H, Fichna J. Role of environmental pollution in irritable bowel syndrome. *World J Gastroenterol* 2015; 21: 11371-11378 [PMID: 26523104 DOI: 10.3748/wjg.v21.i40.11371]
24 Labus JS, Mayer EA, Jarcho J, Kilpatrick LA, Kilkens TO, Evers EA, Backes WH, Brummer RJ, van Nieuwenhoven MA. Acute cypotential deplemet alters the effective connectivity of emotional arousal circuitry during visceral stimuli in healthy women.
Gut 2011; 60: 1196-1203 [PMID: 21420618 DOI: 10.1136/gut.2010.213447]

Camilleri M. Peripheral mechanisms in irritable bowel syndrome. N Engl J Med 2012; 367: 1626-1635 [PMID: 23094724 DOI: 10.1056/NEJMra1107068]

Törnbloom H, Van Oudenhove L, Tack J, Simrén M. Interaction between preprandial and postprandial rectal sensory and motor abnormalities in IBS. Gut 2014; 63: 1441-1449 [PMID: 24142965 DOI: 10.1136/gutjnl-2013-305853]

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.2009.01.018]

Gershon MD. Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterology 2007; 132: 397-414 [PMID: 17241888 DOI: 10.1053/j.gastro.2006.11.002]

Chin A, Svejda B, Gustafsson BI, Granlund AB, Sandvik AK, Timberlake A, Sumpio B, Pfragnek R, Modlin IM, Kidd M. The role of mechanical forces and adenosine in the regulation of intestinal enterochromaffin cell serotonin secretion. Am J Physiol Gastrointest Liver Physiol 2012; 302: G397-G405 [PMID: 22038827 DOI: 10.1152/ajpgi.00087.2011]

Bjerregaard H, Severinson 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]

Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyn H, Crowell MD, Starkey KA, Gershon MD, Maw 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]

Kerckhoffs AP, Ter Linde JJ, Akkermans LM, Samsom M. Trypsinogen IV, serotonin transporter transcript levels and serotonin content are increased in small intestine of irritable bowel syndrome patients. Neurogastroen Motil 2008; 20: 900-907 [PMID: 18363639 DOI: 10.1111/j.1365-2982.2008.01100.x]

Wendelbo I, Mazzawi T, El-Salhy M. Increased serotonin transporter immunoreactivity intensity in the ileum of patients with irritable bowel disease. Mol Med Rep 2014; 9: 180-184 [PMID: 24213511 DOI: 10.3892/mmr.2013.1784]

Faure C, Patey N, Gauhrier C, Brooks EM, Maw 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]

Areeshi MY, Haque S, Panda AK, Mandal RK. A serotonin transporter gene (SLC6A4) polymorphism is associated with reduced risk of irritable bowel syndrome in American and Asian population: a meta-analysis. PLoS One 2013; 8: e75567 [PMID: 24069428 DOI: 10.1371/journal.pone.0075567]

Sandin A, Rangel J, Rispens LJ, Brummer RJ. Cytokine Response after Stimulation with Key Commensal Bacteria Differ in Post-Infectious Irritable Bowel Syndrome (PI-IBS) Patients Compared to Healthy Controls. PLoS One 2015; 10: e0134836 [PMID: 26366730 DOI: 10.1371/journal.pone.0134836]

Goulet O. Potential role of the intestinal microbiota in programming health and disease. Nutr Rev 2015; 73 Suppl 1: S2-40 [PMID: 26175488 DOI: 10.1093/nuture/nuv039]

Sautry A, Mouillet-Richard S, Schneider B, Launay JM, Kellermann O. mir-16 targets the serotonin transporter: a new facet for adaptive responses to antidepressants. Science 2010; 329: 1537-1541 [PMID: 20847275 DOI: 10.1126/science.1193692]

Berger M, Gray JA, Roth BL. The expanded biology of serotonin. Annu Rev Med 2009; 60: 355-366 [PMID: 19630576 DOI: 10.1146/annurev.med.60.042007.110802]
polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome. *Gastroenterology* 2002; 123: 425-432 [PMID: 12145793]

57. *Sarkar JM*, Choi MG, Park JA, Oh JH, Cho YK, Lee IS, Kim SW, Choi KY, Chung IS. Serotonin transporter gene polymorphism and irritable bowel syndrome. *Neurogastroenterol Motil* 2006; 18: 955-1000 [PMID: 17040410 DOI: 10.1111/j.1365-2982.2006.00829.x]

58. *Kohen R*, Jarrett ME, Cain KC, Jun SE, Navaja GP, Symonds S, Heitkemper MM. The serotonin transporter polymorphism rs25531 is associated with irritable bowel syndrome. *Dig Dis Sci* 2009; 54: 2663-2670 [PMID: 19125330 DOI: 10.1007/s10620-008-0666-x]

59. *Camilleri M*, Andrews CN, Bharucha AE, Carlson PJ, Ferber I, Stephens D, Smyrk TC, Urrutia A, Ressens J, Thielemans L, Gohlmann H, van den Wyngaert I, Pouli B. Alterations in expression of p11 and SERT in mucosal biopsy specimens of patients with irritable bowel syndrome. *Gastroenterology* 2007; 132: 17-25 [PMID: 17241856 DOI: 10.1053/j.gastro.2006.11.020]

60. *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/mnm.12133]

61. *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* 2013; 196(11): 2143-9906 DOI: 10.1016/j.coph.2011.02.008

62. *Prasad HC*, Benjamin J, Müller CR, Hamer DH, Murphy DL. Association of serotonin transporter genetic polymorphisms and irritable bowel syndrome. *Gastroenterology* 2015; 148(9): 2585-2595 [PMID: 25511494 DOI: 10.1016/j.gastro.2014.10.092]

63. *Ye A*, Boyd P, Lumsden S, Saunders T, Handley A, Stubbins M, Knaagg A, Asquith S, Taylor I, Bahari B, Crocker N, Rallan R, Varsi S, Montgomery D, Alpers DH, Gules P, Purvis I, Hicks GA. Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women. *Gut* 2004; 53: 1452-1458 [PMID: 15361494 DOI: 10.1136/gut.2003.035451]

64. *Kumar S*, Ranjan P, Mittal B, Ghoshal UC. Serotonin transporter polymorphism (SLC6A4) polymorphism in patients with irritable bowel syndrome and healthy controls. *J Gastroinestin Liver Dis* 2012; 21: 31-38 [PMID: 22457857]

65. *Sikander A*, Rana SV, Sinha SK, Prasad KK, Arora SK, Sharma SK, Singh K. Serotonin transporter promoter variant: Analysis in Indian IBS patients and control population. *J Clin Gastroenterol* 2009; 43: 95-961 [PMID: 19687750 DOI: 10.1097/MCG.0b013e3181b378ec]

66. *Pata C*, Erdal ME, Derici E, Yazar A, Kanik A, Ulu O. Serotonin transporter gene polymorphism in irritable bowel syndrome. *Am J Gastroenterol* 2002; 97: 1780-1784 [PMID: 12135035 DOI: 10.1111/j.1572-0241.2002.00581.x]

67. *Zhang ZF*, Duan ZZ, 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]

68. *Wang BM*, Wang YM, Zhang WM, Zhang QY, Liu WT, Zhang B, Zhang L. The association of serotonin transporter gene polymorphisms and irritable bowel syndrome. *Zhonghua Neu Ke Za Zhi* 2004; 43: 439-441 [PMID: 15312441]

69. *Li Y*, Nie Y, Xie J, Tang W, Liang P, Sha W, Wang H, Zhou Y, Zou H. The association of serotonin transporter gene polymorphisms and irritable bowel syndrome and its influence on tegaserod treatment in Chinese patients. *Dig Dis Sci* 2007; 52: 2942-2949 [PMID: 17394071 DOI: 10.1007/s10620-006-9679-y]

70. *Gill RK*, Kumar A, Malhotra P, Maher D, Singh V, Dudeja PK, Alrefai W, Sakesna S. Regulation of intestinal serotonin transporter expression via epigenetic mechanisms: role of HDAC2. *J Physiol Cell Physiol* 2013; 304: C334-C341 [PMID: 23195070 DOI: 10.1152/jpcell.00361.2012]

71. *Brennecke J*, Stark A, Russell RB, Cohen SM. Principles of microRNA-target recognition. *PLoS Biol* 2005; 3: e85 [PMID: 15723116 DOI: 10.1371/journal.pbio.0030085]

72. *Krol J*, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 2010; 11: 597-610 [PMID: 20661255 DOI: 10.1038/nrg2843]

73. *Barlet DP*. MicroRNAs: target recognition and regulatory functions. *Cell* 2009; 136: 215-233 [PMID: 19167326 DOI: 10.1016/j.cell.2009.01.002]

74. *Liu B*, Li J, Cairns MJ. Identifying miRNAs, targets and functions. *Brief Bioinform* 2014; 15: 1-19 [PMID: 23175680 DOI: 10.1093/bib/bbs075]

75. *Zhang R*, Su B. Small but influential: the role of microRNAs
on gene regulatory network and 3’UTR evolution. *J. Genet Genomics* 2009; 36: 1-6 [PMID: 19161940 DOI: 10.1016/S1673-8529(09)60001-1]

87 Croce CM. Causes and consequences of microRNA dysregulation in cancer. *Nat Rev Genet* 2009; 10: 704-714 [PMID: 19763153 DOI: 10.1038/nrg2634]

88 Millan MJ. MicroRNA in the regulation and expression of serotonergic transmission in the brain and other tissues. *Curr Opin Pharmacol* 2011; 11: 11-22 [PMID: 21345728 DOI: 10.1016/j.coph.2011.01.008]

89 Yue J, Tiggy G. Conservation of miR-15a-16 and miR-15b-16-2 clusters. *Mamm Genome* 2010; 21: 88-94 [PMID: 20013340 DOI: 10.1007/s00494-010-2420-3]

90 Song MF, Dong JZ, Wang YW, He J, Xu X, Zhang L, Zhang YH, Shi JF, Lv YY. CSF miR-16 is decreased in major depression patients and its neutralization in rats induces depression-like behaviors via a serotonin transporter system. *J. Affect Disord* 2015; 178: 25-31 [PMID: 25779937 DOI: 10.1016/j.jad.2015.02.022]

91 Launay JM, Mouillet-Richard S, Baudry A, Pietri M, Kellermann O. Raphe-mediated signals control the hippocampal response to SRI antidepressants via miR-16. *Transl Psychiatry* 2011; 1: e56 [PMID: 22833211 DOI: 10.1038/tp.2011.54]

92 Dwivedi Y. Evidence demonstrating role of microRNAs in the etiopathology of major depression. *J. Chem Neuroanat* 2011; 42: 142-156 [PMID: 21515361 DOI: 10.1016/j.jchemneu.2011.04.002]

93 Tamarapu Parthasarathy P, Galar M, Hyunh B, Yunus A, Abuelenen T, Castillo A, Kollongod Ramanathan G, Cox R, Kolliputi N. MicroRNA-16 modulates epithelial sodium channel in human alveolar epithelial cells. *Biochem Biophys Res Commun* 2012; 420: 263-268 [PMID: 22940131 DOI: 10.1016/j.bbrc.2012.08.063]

94 Jensen KP, Kovault J, Conner TS, Tennen H, Kranzler HR, Furneaux HM. A common polymorphism in serotonin receptor 1B mRNA regulates modulation by mirt-96 and associates with aggressive human behaviors. *Mol Psychiatry* 2009; 14: 381-389 [PMID: 18283276 DOI: 10.1038/mp.2008.15]

95 Sivamurthi BS, Madhumita R, Balamurugan K, Rajan KE. Cronobacter sakazakii infection alters serotonin transporter and improved fear memory retention in the rat. *Front. Microbiol* 2015; 6: 188 [PMID: 26838777 DOI: 10.3389/fmicb.2015.00188]

96 Liao XJ, Mao WM, Wang Q, Yang GG, Wu WJ, Shao SX. MicroRNA-24 inhibits serotonin reuptake transporter expression and aggravates irritable bowel syndrome. *Biochem Biophys Res Commun* 2016; 469: 288-293 [PMID: 26631964 DOI: 10.1016/j.bbrc.2016.01.025]

97 Spiller RC, Lam C, An Update on Post-inflammatory Irritable Bowel Syndrome: Role of Genes, Immune Activation, Serotonin and Altered Microbiome. *J Neurogastroenterol Motil* 2012; 18: 258-268 [PMID: 22837873 DOI: 10.5056/jgmm.2012.18.3.258]

98 Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner LA, Saarela M, Korpela R, Palva A. Analysis of the fecal microbiota composition and host-microbe cross-talk following resection in post-infectious irritable bowel syndrome: gender-dependence and association with digestive symptoms. *Am J Gastroenterol* 2009; 104: 392-400 [PMID: 19174979 DOI: 10.1038/ajg.2008.94]

99 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. *Neuropsychopharmacol Motil* 2005; 17: 863-870 [PMID: 16336502 DOI: 10.1111/j.1665-2782.2005.00719.x]

100 Foley KF, Pantano C, Ciolino A, Maw G. 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: 17107025 DOI: 10.1152/ajpgi.00470.2006]

101 Shi HL, Liu CH, Ding LL, Zheng Y, Fei XY, Lu L, Zhou XM, Yuan JY, Xie JQ. Alterations in serotonin, transient receptor potential channels and protease-activated receptors in rats with irritable bowel syndrome attenuated by Shugan decoction. *World J Gastroenterol* 2015; 21: 4852-4863 [PMID: 25944998 DOI: 10.3748/wjg.v21.i16.4852]

102 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]

103 Distretti E, Monaldi L, Ricci P, Fiorucci S. Gut microbiota role in irritable bowel syndrome: New therapeutic strategies. *World J Gastroenterol* 2016; 22: 2219-2244 [PMID: 26900286 DOI: 10.3748/wjg.v22.i17.2219]

104 Jalanka J, Salonen A, Fuentes S, de Vos WM. Microbial signatures in post-inflammatory irritative bowel syndrome--toward patient stratification for improved diagnostics and treatment. *Gut Microbes* 2015; 6: 364-369 [PMID: 26512631 DOI: 10.1080/19490063.2015.1096486]

105 Simrén M, Barbara G, Flijt HQ, Spiegel BM, Spiller RC, Vanner S, Verdu EF, Whorwell PJ, Zoetendal EG. Intestinal microbiota in functional bowel disorders: a Rome Foundation report. *Gut* 2013; 62: 159-176 [PMID: 22730468 DOI: 10.1136/gut.2012-320167]

106 Jalanka-Tuovinen J, Salojärvi J, Salonen A, Immonen O, Garsed K, Kelly FM, Zaitoun A, Palva A, Spiller RC, de Vos WM. Faecal microbiota composition and host-microbe cross-talk following faecal reinfec tion and in postinfectious irritable bowel syndrome. *Gut* 2014; 63: 1737-1745 [PMID: 24310267 DOI: 10.1136/gutjnl-2013-305994]

107 Ringel Y, Ringel-Kulka T. The Intestinal Microbiota and Irritable Bowel Syndrome. *J Clin Gastroenterol* 2015; 49 Suppl 1: S56-S59 [PMID: 26447966 DOI: 10.1097/MCG.0000000000000418]

108 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]

109 Krogulis-Kurikka L, Lyra A, Malinen E, Aamikunnas J, Tuimala J, Paulin L, Mäkivuoekko 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. *BMJ Gastroenterol* 2009; 9: 95 [PMID: 20015409 DOI: 10.1016/S1742-230X(9)-9]

110 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]
Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, Nagler CR, Ismagilov RF, Mazmanian SK, Hsiao EY. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell 2015; 161: 264-276 [PMID: 25860609 DOI: 10.1016/ j. cell.2015.02.029]

Reigstad CS, Salmonson CE, Rainey JF, Szurszewski JH, Linden DR, Sonnenburg JL, Farruggia G, Kashyap PC. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J* 2015; 29: 1395-1403 [PMID: 25550456 DOI: 10.1096/f.14-259598]

Esmaili A, Nazir SF, Borthakur A, Yu D, Turner JR, Saksena N, Singla A, Hecht GA, Alrefai WA, Gill RK. Enteropathic gene expression in human glial cells by growth factors. *Eur J Pharmacol* 2001; 417: 69-76 [PMID: 11301061]

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

Carpenter G, Cohen S. Epidermal growth factor. *J Biol Chem* 1990; 265: 7709-7712 [PMID: 2186024]

Dvorak B. Milk epidermal growth factor and gut protection. *J Pediatr* 2010; 156: S31-S35 [PMID: 20150663 DOI: 10.1016/j. jpeds.2009.11.018]

Niederlehner S, Baird C, Petrie B, Wischmeyer E, Wischmeyer PE. Epidermal growth factor receptor expression and signaling are essential in glutamine’s cytoprotective mechanism in heat-stressed intestinal epithelial-6 cells. *Am J Physiol Gastrointest Liver Physiol* 2013; 304: G543-G552 [PMID: 23725616 DOI: 10.1152/jappl.00418.2012]

Danielsen AJ, Maithe NJ. The EGF/ErbB receptor family and apoptosis. *Growth Factors* 2002; 20: 1-15 [PMID: 11999214]

Ménard D, Corriveau L, Arsenault P. Differential effects of epidermal growth factor and hydrocortisone in human fetal colon. *J Pediatr Gastroenterol Nutr* 2010; 10: 13-20 [PMID: 2248747]

Carpenter G. Epidermal growth factor is a major growth-promoting agent in human milk. *Science* 1980; 210: 198-199 [PMID: 6968093]

Miettinen PJ, Berger JE, Meneses J, Phung Y, Pedersen RA, Werb Z, Derynck R. Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. *Nature* 1995; 376: 337-341 [PMID: 1038373370]

Fragus S, Barberán S, Cebriá F. EGF signaling regulates cell proliferation, differentiation and morphogenesis during planarian regeneration and homeostasis. *Dev Biol* 2011; 354: 87-101 [PMID: 21458439 DOI: 10.1016/j.ydbio.2011.03.023]

Jones MK, Tomikawa M, Mohajer B, Tarnawski AS. Gastrointestinal mucosal regeneration: role of growth factors. *Front Biosci* 1999; 4: D374-D390. [PMID: 10077546]

Gill RK, Anbazhagan AN, Esmaili A, Kumar A, Nazir S, Malakooti J, Alrefai WA, Saksena S. Epidermal growth factor upregulates serotonin transporter in human intestinal epithelial cells via transcriptional mechanisms. *Am J Physiol Gastrointest Liver Physiol* 2011; 300: G627-G636 [PMID: 21273531 DOI: 10.1152/jappl.00563.2010]

Linden DR, White SL, Brooks EM, Mawe GM. Novel promoter and alternate transcription start site of the human serotonin reuptake transporter in intestinal mucosa. *Neurogastroenterol Motil* 2009; 21: 534-541, e10-11 [PMID: 19227758 DOI: 10.1111/j.1365-2982.2008.01247.x]

Cui XF, Zhou WM, Yang Y, Zhou J, Li XL, Lin L, Zhang HJ. Epidermal growth factor upregulates serotonin transporter and its association with visceral hypersensitivity in irritable bowel syndrome. *World J Gastroenterol* 2014; 20: 13521-13529 [PMID: 25309082 DOI: 10.3748/wjg.v20.i37.13521]

Willot S, Gauthier C, Patey N, Faure C. Nerve growth factor content is increased in the rectal mucosa of children with diarrheaa-predominant irritable bowel syndrome. *Neurogastroenterol Motil* 2012; 24: 734-739, e347 [PMID: 22625872 DOI: 10.1111/j.1365-2982.2012.01933.x]

Dothel G, Barbaro MR, Boudin H, Vasina V, Cremon C, Gargano L, Bellacosa L, De Giorgio R, Le Berre-Scoul C, Aubert P, Neunlist M, De Ponti F, Stanghellini V, Barbara G. Nerve fiber outgrowth is
increased in the intestinal mucosa of patients with irritable bowel syndrome. *Gastroenterology* 2015; **148**: 1002-1011.e4 [PMID: 25655556 DOI: 10.1053/j.gastro.2015.01.042]

*Matricon J*, Muller E, Accarie A, Meleine M, Etienne M, Voilley N, Bussereilles J, Eschalier A, Lazdunski M, Bourdu S, Gelet A, Arbier D. Peripheral contribution of NGF and ASIC1a to colonic hypersensitivity in a rat model of irritable bowel syndrome. *Neurogastroenterol Motil* 2013; **25**: e740-e754 [PMID: 23902154 DOI: 10.1111/amo.12199]

*Xu XJ*, Liu L, Yao SK. Nerve growth factor and diarrhea-predominant irritable bowel syndrome (IBS-D): a potential therapeutic target? *J Zhejiang Univ Sci B* 2016; **17**: 1-9 [PMID: 26739521 DOI: 10.1631/jzus.B1500181]

*Zimmermann K*, van Phi VD, Brase A, Phi-van L. Inhibition of serotonin transporter expression by C/EBPβ in LPS-activated macrophage cells (HD11). *Innate Immun* 2015; **21**: 406-415 [PMID: 25213348 DOI: 10.1177/1753425914547434]

*Yoon Y*, McKenna MC, Rollins DA, Song M, Nuriel T, Gross SS, Xu G, Glatt CE. Anxiety-associated alternative polyadenylation of the serotonin transporter mRNA confers translational regulation by hnRNP-K. *Proc Natl Acad Sci USA* 2013; **110**: 11624-11629 [PMID: 23798440 DOI: 10.1073/pnas.1301485110]

*Giannaccini G*, Betti L, Palego L, Schmid L, Fabbrini L, Pelosini C, Gargi C, Da Valle Y, Lanza M, Marsili A, Maffei M, Santini F, Vitti P, Pinchera A, Lucacchini A. Human serotonin transporter expression during megakaryocytic differentiation of MEG-01 cells. *Neurochem Res* 2010; **35**: 628-635 [PMID: 20041293 DOI: 10.1007/s11064-009-0112-8]
