

Effects of regulating intestinal microbiota on anxiety symptoms: A systematic review

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ABSTRACT

Background Anxiety symptoms are common in mental diseases and a variety of physical disorders, especially in disorders related to stress. More and more basic studies have indicated that gut microbiota can regulate brain function through the gut-brain axis, and dysbiosis of intestinal microbiota was related to anxiety. However, there is no specific evidence to support treatment of anxiety by regulating intestinal microbiota.

Aims To find evidence supporting improvement of anxiety symptoms by regulation of intestinal microbiota.

Methods This systematic review of randomised controlled trials was searched based on the following databases: PubMed, EMBASE, the Cochrane Library, OVID, Web of Knowledge, China National Knowledge Infrastructure (CNKI), Wanfang Data, VIP databases and SinoMed. The retrieval time dated back to 25 July 2018. Then we screened research literatures based on established inclusion and exclusion criteria. Quality evaluation for each included study was done using the Cochrane risk of bias and the Jadad scale.

Results A total of 3334 articles were retrieved and 21 studies were included which contained 1503 subjects. In the 21 studies, 14 chose probiotics as interventions to regulate intestinal microbiota and six chose non-probiotic ways such as adjusting daily diets. Probiotic supplements in seven studies contained only one kind of probiotic, two studies used a product that contained two kinds of probiotics and the supplements used in the other five studies included at least three kinds of probiotics. In the studies that used treatment as usual plus interventions regulating intestinal flora (IRIF) as interventions (five studies), only non-probiotic ways were effective (two studies), which means 40% of studies were effective; in the studies that used IRIF alone (16 studies, 11 studies used probiotic ways and 5 studies used non-probiotic ways), 56% of studies could improve anxiety symptoms, and 80% of studies that conducted the non-probiotic interventions were effective, while 45% of studies that used probiotic supplementations had positive effects on anxiety symptoms. Overall, 11 studies showed a positive effect on anxiety symptoms by regulating intestinal microbiota, which indicated 52% of the 21 studies were effective, and there were five studies that used probiotic supplements as interventions and six used non-probiotic interventions. In addition, it should be noted that six of seven studies showed that regulation of intestinal microbiota could treat anxiety symptoms, the rate of efficacy was 86%.

Conclusions We find that more than half of the studies included showed it was positive to treat anxiety symptoms by regulation of intestinal microbiota. There are two kinds of interventions (probiotic and non-probiotic interventions) to regulate intestinal microbiota, and it should be highlighted that the non-probiotic interventions were more effective than the probiotic interventions. More studies are needed to clarify this conclusion since we still cannot run meta-analysis so far.

BACKGROUND

Anxiety disorder is a mental disorder with anxiety symptoms as the main clinical manifestation, with a global incidence of 3%–25%, and the incidence in chronic diseases, such as cancer, cardiocerebrovascular disease, irritable bowel syndrome (IBS), is 1.4%–70%.¹ Studies² have shown that up to 33.7% of people will be affected by anxiety symptoms during their lifetime. Those with a longer course of disease are often accompanied by social cognitive impairment, which has serious impact on patients and society. Therefore, the treatment of anxiety is very important. Clinical principles for the treatment of physical diseases with anxiety symptoms are usually based on the relief of somatic symptoms, and the use of psychiatric drugs, psychotherapy and other treatments can be combined under the premise of ensuring treatment efficacy. In China, anxiety symptoms often are confused with somatic symptoms and neglected in clinical practice.³ Therefore, the anxiety symptoms often could not be treated timely and effectively.

The trillions of microorganisms located in the gut are called gut microbiota, and they perform important functions in the immune system and metabolism by providing essential inflammatory mediators, nutrients and vitamins.⁴ Besides, Toll-like receptors (TLR) can specifically recognise lipopolysaccharide (LPS) molecules in pathogenic microorganisms, especially TLR4. After the LPS of the gut microbiota activates the TLR, the NF- κ B pathway which regulates the expressions



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of many inflammatory mediators and cytokines is activated. The long-term existence of this immune activation can make brain functions change which finally lead to the kinds of mental disorders like anxiety disorder.⁵⁻⁷ Furthermore, studies indicated that gut microbiota could have an impact on the function of the hypothalamus-pituitary-adrenal axis which could lead to changes in brain functions.⁸ Additionally, a growing number of basic and clinical studies have shown that intestinal flora can modulate communication between the gut and the brain⁹ via the gut-brain axis, which¹⁰ mainly includes the nervous system, immune system and endocrine system. When intestinal flora is affected, a series of changes in physical and/or mental symptoms can occur.¹¹

Animal studies have demonstrated that germ-free mice pretended to have anxiety-related behaviours and this condition could be changed by regulating gut microbiota.¹²⁻¹⁶ However, there is no consensus on whether anxiety symptoms can be improved by regulating gut microbiota. Therefore, this systematic review was conducted to provide clarification and new ideas for clinical treatment.

METHODS

Search strategy

The following databases were searched up to 25 July 2018: PubMed, EMBASE, the Cochrane Library, OVID, Web of Knowledge, China National Knowledge Infrastructure (CNKI), Wanfang Data, VIP Databases and SinoMed. The search terms were as follows: (anxiety OR anxiety disorder OR generalized anxiety disorder OR GAD OR social anxiety disorder OR SAD) AND (intestinal microbiota OR gut bacteria OR enteric microbiome OR gut microbiota OR fecal microbiota OR intestinal flora OR gut flora). The Chinese search terms were “焦虑” or “焦虑症状”, AND “肠道菌群”. The retrieval strategy and keywords were modified for different databases. At the same time, we conducted literature traceability to find further relevant research.

Inclusion and exclusion criteria of literature

Inclusion criteria

The inclusion criteria were as follows: (1) patients: the subjects were patients with anxiety symptoms no matter what the diagnoses were and all patients should have been assessed by at least one kind of anxiety scale; (2) interventions versus comparisons: (A) treatment as usual (TAU) plus interventions regulating intestinal flora (IRIF, such as the supplementary of probiotic, changing diet habits, and so on) versus TAU, (B) IRIF alone versus placebo; (3) outcomes: the main outcome of the study was the anxiety symptom measured by kinds of anxiety assessment scales, such as the Hospital Anxiety and Depression Scale (HADS), the Beck Anxiety Inventory (BAI), the State-Trait Anxiety Inventory (STAI), and so on; (4) studies: the study must be a clinical randomised controlled study.

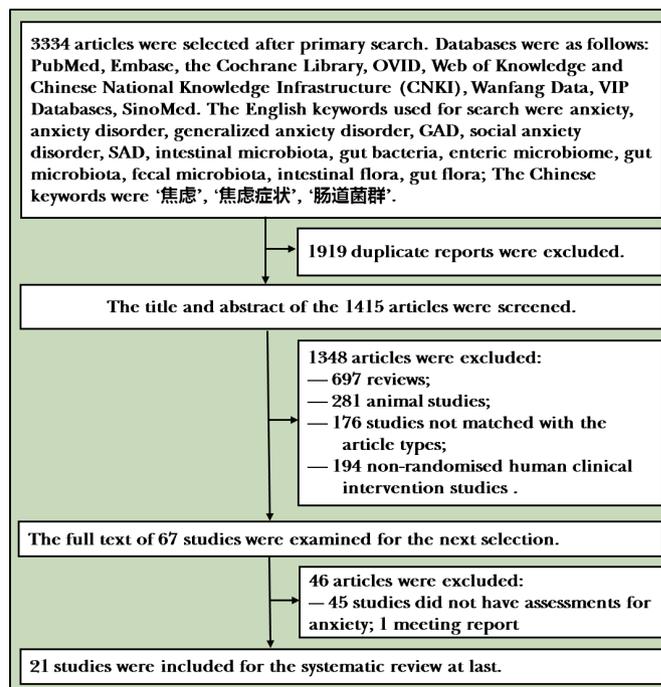


Figure 1 Flowchart of the study. 21 studies were selected for the systematic review after retrieving the databases based on the search strategy.

Exclusion criteria

The exclusion criteria were: (1) non-clinical randomised controlled trials; (2) non-human studies; (3) reviews; (4) study protocols; (5) data incomplete experiments like meeting reports and so on; and (6) repeated reports.

Literature screening and data extraction

The literature screening process of this study is shown in figure 1. The literature search was performed independently by two researchers (B-BY, J-BW) according to the search strategy. If the two search results were different, the two researchers reviewed the literature together and analysed the reasons for the differences. If the opinions were still inconsistent, a third person (P-JJ) would examine and make the final decision. If there was a lack of information in the literature, it was supplemented by contacting the author. We developed the data extraction table for data extraction and verification, and extraction contents included (A) basic information for research; (B) methods of included research; (C) subjects; and (D) interventions and outcomes.

Quality evaluation of literature

Risk of bias evaluation: the included studies were assessed by two independent investigators based on the risk of bias assessment method recommended by the Cochrane manual version 5.3.0. The specific contents included: (A) random sequence generation; (B) allocation concealment; (C) whether to use the blind method (blinding of the subjects and the treatment providers, blinding of the result evaluators); (D) incomplete results data; and (E) other potential risks affecting authenticity. When there

were differences between the two evaluators, a third person would make the decision.

Evidence quality assessment : The scoring standards of Jadad scale were as follows: (A) randomisation: (1) the method of randomisation was described and it was appropriate (two points), (2) the study was described as randomised (one point), (3) not randomised or inappropriate method of randomisation (zero point); (B) concealment of allocation: (1) the method of allocation concealment was described appropriately (two points), (2) the study was described as using allocation concealment method (one point), (3) did not describe the method of allocation concealment (zero point); (C) double blinding: (1) the method of double blinding was described and it was appropriate (two points), (2) the study was described as double blind (one point), (3) no blind or inappropriate method of blinding (zero point); (D) withdrawals and dropouts: (1) a description of withdrawals and dropouts (one point), (2) did not describe the follow-up (zero point). One to three points is considered low quality and four to seven points as high quality.

RESULTS

Basic characteristics of the included literature

The research process is shown in [figure 1](#). A total of 3334 studies were included after retrieving articles from five English databases and four Chinese databases based on the search strategy, and the released deadline of the studies was 25 July 2018. First, 1919 unrelated studies were removed, with 1415 studies remaining in the secondary step. After reading the titles and summaries, 1348 articles were excluded. Finally, after reading the remaining 67 articles, 46 were removed (45 lacking assessments of anxiety and 1 was a meeting report) with 21 studies remaining for the systematic evaluation.

The details of the 21 papers included are shown in [table 1](#). A total of 1503 subjects were included in the 21 studies, including patients with IBS (10 studies), healthy controls (six studies) and other patients with chronic diseases such as: chronic fatigue syndrome (CFS), rheumatoid arthritis (RA), obesity, fibromyalgia and type 2 diabetes mellitus. Five studies conducted TAU plus IRIF when the TAU did not affect the results: three studies used a single kind of probiotic as interventions and two studies conducted non-probiotic ways (supplementary of the resistant dextrin or a diet low in fermentable oligosaccharides, disaccharides, and monosaccharides and polyols [low FODMAP]). The studies that used IRIF alone (16 studies) could be divided into two categories: (1) probiotic interventions (11 studies): (A) single probiotic interventions (four studies), and most of the probiotics were *Lactobacillus*, (B) two studies used two probiotic mixtures: the Swiss *Lactobacillus* and the long *Bifidobacterium* mixture, (C) five studies used at least three probiotic mixtures: *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, and so on; (2) five studies conducted non-probiotic interventions, including low FODMAP, short-chain

fructooligosaccharides (scFOS), regulating diet, using *trans*-galactooligosaccharide mixture, and so on. The most used questionnaires for assessing anxiety symptoms included the HADS (nine studies), the STAI (seven studies), the BAI (two studies), Hamilton Anxiety Rating Scale (HAM-A; two studies), and so on, with five studies choosing two different scales: Sawada and colleagues¹⁷ and Pinto-Sanchez and colleagues¹⁸ used HAD and STAI; Messaoudi and colleagues¹⁹ used HAD and the Hopkins Symptom Checklist-90; Kelly and colleagues²⁰ used STAI and BAI; Farhangi and colleagues²¹ used HAM-A and a 42-item self-report questionnaire designed to assess the current severity of symptoms relating to depression, anxiety and stress (Depression, Anxiety and Stress Scale-42). Except for Schumann and colleagues²² choosing yoga as the intervention in control group, the interventions of the other studies in the control group were matched with the experimental group. All the supplements could not be distinguished by appearance and taste which ensured the blindness of the subjects.

Research quality

The results of the quality assessment are shown in [table 2](#). Of all the 21 studies, only Sanchez and colleagues²³ did not mention methods of random sequence generation, resulting in a rating of 'unclear', and the other studies were all rated as 'low'. According to the Jadad scale, 81% of the included studies were ≥ 4 points and assessed as high quality. In addition, 33% of the studies conducted intent-to-treat analysis in order to maintain the random information, ensuring the equilibrium between the groups. Seventeen studies mentioned the rate of withdrawal and/or dropout, which were $\leq 20\%$ (the reasons are shown in [table 3](#)). In summary, the overall quality of the 21 articles included in this study was high.

Therapeutic effects

Eleven of 21 studies showed that regulation of intestinal microbiota could improve anxiety symptoms, of which five studies conducted probiotic interventions and six studies used non-probiotic interventions like low FODMAP. That means that 52% of studies showed a positive effect on improving anxiety symptoms by regulating intestinal microbiota, seen in [figure 2](#). In the five studies that used TAU plus IRIF, the anxiety symptoms were improved all by non-probiotic ways (two studies) while the other three studies that used one kind of probiotic were all invalid. So, we could find that 40% of studies that used TAU plus IRIF were positive and 56% of studies that used IRIF alone could improve anxiety symptoms. Anxiety assessment questionnaires included HADS, STAI, BAI and HAM-A. This also indicated that no matter what kinds of measures were taken, and regardless of the assessment scales, anxiety symptoms could be improved by regulating gut microbiota. It is worth mentioning that the efficiency of supplementation of non-probiotic preparations is as high as 86%, which suggested that in addition to supplementing probiotics, it is worth noticing by clinicians to

Table 1 Basic characteristics of the included literature

ID	Author	Year	Methods	Subjects			Interventions	Scales
				Diagnosis	Sample size (n)	Interventions		
1	Roman <i>et al</i> ²⁶	2018	Randomised double blind	Fibromyalgia	31	Three probiotic mixtures or above	STAI	
2	Farhangi <i>et al</i> ²¹	2018	Randomised triple blind	T2DM*	62	Resistant dextrin†	GHQ, DASS	
3	Schumann <i>et al</i> ²²	2018	Randomised single blind	IBS	59	Low FODMAP	HADS	
4	Sawada <i>et al</i> ^{17, ‡}	2017	Randomised double blind	Healthy individuals§	24	<i>Lactobacillus gasseri</i>	HADS , STAI	
5	Sanchez <i>et al</i> ²³	2017	Randomised double blind	Obesity	105	<i>Lactobacillus rhamnosus</i>	STAI	
6	Romijn <i>et al</i> ²⁷	2017	Randomised double blind	IBS	79	Two probiotic mixtures†	DASS	
7	Pinto-Sanchez <i>et al</i> ¹⁸	2017	Randomised double blind	IBS	44	<i>Bifidobacterium longum</i>	HADS , STAI	
8	Kelly <i>et al</i> ^{20, ‡}	2017	Randomised	Healthy individuals§	29	<i>L. rhamnosus</i>	BAI, STAI	
9	Eswaran <i>et al</i> ²⁸	2017	Randomised single blind	IBS	84	Low FODMAP†	HADS	
10	Colica <i>et al</i> ²⁹	2017	Randomised	Healthy individuals	30	At least three probiotic mixtures	HAM-A	
11	Azpiroz <i>et al</i> ³⁰	2017	Randomised double blind	IBS	79	scFOS	HADS	
12	Lyra <i>et al</i> ³¹	2016	Randomised triple blind	IBS	340	<i>Lactobacillus acidophilus</i> †	HADS	
13	Steenbergen <i>et al</i> ³²	2015	Randomised triple blind	Healthy individuals	89	At least three probiotic mixtures	BAI	
14	Lorenzo-Zúñiga <i>et al</i> ³³	2014	Randomised triple blind	IBS	84	At least three probiotic mixtures	VSI	
15	Peters <i>et al</i> ^{34, ‡}	2014	Randomised double blind	IBS	22	Supplementation of gluten	STPI	
16	Alipour <i>et al</i> ³⁵	2014	Randomised double blind	RA*	46	<i>Lactobacillus casei</i> †	STAI	
17	Yuman and Yingwei ³⁶	2013	Randomised double blind	Healthy individuals	82	Nutritional interventions	HAM-A	
18	Messaoudi <i>et al</i> ¹⁹	2011	Randomised double blind	Healthy individuals	55	Two probiotic mixtures	HADS HSCL-90	
19	Simrén <i>et al</i> ³⁷	2010	Randomised double blind	IBS	74	At least three probiotic mixtures	HADS	
20	Silk <i>et al</i> ^{38, ‡}	2009	Randomised single blind	IBS	44	<i>Trans-galactooligosaccharide</i> mixture	HADS	
21	Rao <i>et al</i> ³⁹	2009	Randomised double blind	CFS	39	<i>L. casei</i>	BAI	

DASS-42 refers to the 42-item self-report questionnaire designed to assess current severity of symptoms relating to depression, anxiety and stress.

*All the subjects were female.

†Studies conducted treatment as usual (TAU) plus interventions regulating intestinal flora (IRIF) interventions.

‡Cross-over study design.

§All the subjects were male.

BAI; Beck Anxiety Inventory; CFS, chronic fatigue syndrome; DASS-42, Depression, Anxiety and Stress Scale; GHQ, General Health Questionnaire; HADS, Hospital Anxiety and Depression Scale; HAM-A, Hamilton Anxiety Rating Scale; HSCL-90, Hopkins Symptom Checklist; IBS, irritable bowel syndrome; RA, rheumatoid arthritis; STAI, State-Trait Anxiety Inventory; STPI, the State Trait Personality Inventory; T2DM, type 2 diabetes mellitus; VSI, Visceral Sensitivity Index; low FODMAP; diet low in fermentable oligosaccharides, disaccharides, and monosaccharides and polyols; scFOS, short-chain fructooligosaccharides.

Table 2 Evaluation of literature quality

The Cochrane risk of bias assessment											
ID	Author	Year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias	Jadad scoring	ITT
1	Roman <i>et al</i> ²⁶	2018	Low	Low	Low	Unclear	Low	Low	Low	7	No
2	Farhangi <i>et al</i> ²¹	2018	Low	Low	Low	Unclear	Low	Low	Low	5	No
3	Schumann <i>et al</i> ²²	2018	Low	High	High	Low	Low	Unclear	Unclear	5	Yes
4	Sawada <i>et al</i> ¹⁷	2017	Low	Unclear	Unclear	Unclear	Unclear	Unclear	Low	3	No
5	Sanchez <i>et al</i> ²³	2017	Unclear	Unclear	Unclear	Unclear	Low	Unclear	Low	4	No
6	Romijn <i>et al</i> ²⁶	2017	Low	Low	Low	Unclear	Low	Unclear	Low	7	Yes
7	Pinto-Sanchez <i>et al</i> ¹⁸	2017	Low	Low	Low	Unclear	Low	Unclear	Low	7	Yes
8	Kelly <i>et al</i> ²⁰	2017	Low	Unclear	Low	Unclear	Unclear	Unclear	Unclear	3	No
9	Eswaran <i>et al</i> ²⁸	2017	Low	Unclear	High	Low	Unclear	Unclear	Unclear	2	Yes
10	Colica <i>et al</i> ²⁹	2017	Low	Unclear	Unclear	Unclear	Low	Unclear	Unclear	3	No
11	Azpiroz <i>et al</i> ³⁰	2017	Low	Unclear	Unclear	Unclear	Low	Unclear	Low	5	Yes
12	Lyra <i>et al</i> ³¹	2016	Low	Low	Low	Low	Low	Unclear	Unclear	7	Yes
13	Steenbergen <i>et al</i> ³²	2015	Low	Unclear	Unclear	Unclear	Low	Unclear	Unclear	4	No
14	Lorenzo-Zúñiga <i>et al</i> ³³	2014	Low	Low	Low	Unclear	Low	Unclear	Unclear	6	No
15	Peters <i>et al</i> ³⁴	2014	Low	Unclear	Unclear	Low	Low	High	Unclear	5	No
16	Alipour <i>et al</i> ³⁵	2014	Low	Low	Low	Unclear	Low	Unclear	Unclear	7	No
17	Yuman and Yingwei ³⁶	2013	Low	Low	Low	Unclear	Unclear	Unclear	Unclear	6	No
18	Messaoudi <i>et al</i> ¹⁹	2011	Low	Low	Low	Unclear	Low	Unclear	Unclear	7	No
19	Simrén <i>et al</i> ³⁷	2010	Low	Low	Low	Low	Low	Unclear	Unclear	7	Yes
20	Silk <i>et al</i> ³⁸	2009	Low	Unclear	Unclear	Unclear	Low	Unclear	Unclear	5	No
21	Rao <i>et al</i> ³⁹	2009	Low	Unclear	Low	Unclear	Low	Unclear	Unclear	5	No

ITT, intent to treat.

Table 3 Adverse effects and reasons for withdrawal and/or dropout

ID	Author	Year	Adverse events (n)	Reasons for withdrawal	
				Intervention group (n)	Control group (n)
1	Roman <i>et al</i> ²⁶	2018	Intestinal discomfort (2)	Reasons unrelated to the intervention (2)	Non-therapeutic adherence (2)
2	Farhangi <i>et al</i> ²¹	2018	No serious adverse events	Did not consume the supplement (1)	Received anti-inflammatory medication (2), diet change (1), did not consume the supplement (3)
3	Schumann <i>et al</i> ²²	2018	FODMAP group: a major depressive episode (1), a mild self-reported depressive episode (1), unwanted loss of weight (1) Yoga group: a newly diagnosed deep leg vein thrombosis (1), back pain (1)	Due to the adverse events (1), scheduling problems (1), compliance (2)	Loss of interest (1), scheduling problems (2)
4	Sawada <i>et al</i> ¹⁷	2017	Abdominal pain, sleep disturbance, particularly in the placebo group	No data provided	
5	Sanchez <i>et al</i> ²³	2017	No data provided	Poor compliance to the treatment (1)	
6	Romijn <i>et al</i> ²⁷	2017	Dry mouth, sleep disruption	Antibiotic use (1), antidepressant use (2), participant choice (4)	Antibiotic use (2), stressful life events (1)
7	Pinto-Sanchez <i>et al</i> ¹⁸	2017	No serious adverse events related to study product	Antibiotic use (3), antidepressant use (1)	Antibiotic use (1), antidepressant use (1)
8	Kelly <i>et al</i> ²⁰	2017	Side effects were negligible	No data provided	
9	Eswaran <i>et al</i> ²⁸	2017	No data provided	Lost to follow-up: not returning calls (1), started antibiotics (1), too expensive (1); discontinued intervention: too limiting (1), moved out of state (1)	Discontinued intervention: failed to make symptom reports (2)
10	Colica <i>et al</i> ²⁹	2017	No data provided	2 subjects voluntarily stopped the treatment	1 subject voluntarily stopped the treatment
11	Azpiroz <i>et al</i> ³⁰	2017	Intake of scFOS was well tolerated and the number of adverse events was similar in the scFOS (18) and placebo (21) groups†	Colonic lavage prior to the rectal sensitivity test (1), antibiotic treatment (1)	
12	Lyra <i>et al</i> ³¹	2016	Treatment-emergent AEs: GI disorders, gastroenteritis and influenza IP-related AEs: mild GI symptoms (7 placebo, 7 low dose and 9 high dose) Two SAE cases: pneumonia and syncope; neither was associated with the IP or any trial procedure	Low dose—adverse event (4), lost to follow-up (3), other (4), protocol violation (1), withdrawal of consent (5) High dose—adverse event (10), other (3), protocol violation (1), withdrawal of consent (4)	Adverse event (3), lost to follow-up (2), other (2), protocol violation (3), withdrawal of consent (6)
13	Steenbergen <i>et al</i> ³²	2015	No data provided	No data provided	
14	Lorenzo-Zúñiga <i>et al</i> ³³	2014	No adverse drug reactions	High dose: loss to follow-up (3), discontinued intervention (3) Low dose: loss to follow-up (3), discontinued intervention (3)	Loss to follow-up (5), discontinued intervention (5)
15	Peters <i>et al</i> ³⁴	2014	No data provided‡	No data provided	
16	Alipour <i>et al</i> ³⁵	2014	No adverse effects	Did not follow the study protocol (8/6)	
17	Yuman and Yingwei ³⁶	2013	No data provided	No data provided	

Continued

Table 3 Continued

ID	Author	Year	Adverse events (n)	Reasons for withdrawal	
				Intervention group (n)	Control group (n)
18	Messaoudi <i>et al</i> ¹⁹	2011	No data provided	No data provided	
19	Simrén <i>et al</i> ³⁷	2010	No adverse events	Lack of effect of the treatment (5), factors unrelated to the study (2)	
20	Silk <i>et al</i> ³⁸	2009	Moderate diarrhoea (1–3.5 g placebo), mild nausea (1–7.0 g placebo, 1–3.5 g prebiotic)	Felt the study was too demanding (3), felt the placebo caused diarrhoea (1), took part in another probiotic study (1), took a commercially available probiotic preparation during baseline (2)	
21	Rao <i>et al</i> ³⁹	2009	No significant adverse events	Reasons unrelated to the intervention (4)	

GI disorders refer to gastrointestinal tract disorders, including abdominal discomfort, abdominal distension, abdominal pain, constipation, diarrhoea and flatulence.

*None of these events were adjudged to relate to the study interventions.

†Did not provide details.

‡One patient ceased the whey challenge (treatment first received) prematurely because of intolerable symptoms after lunch on day 2. AE, adverse effect; FODMAP, fermentable oligosaccharides, disaccharides, and monosaccharides and polyols; GI, gastrointestinal ; IP, investigational product; SAE, serious adverse event; scFOS, short-chain fructooligosaccharides.

improve the anxiety symptoms by regulating the intestinal flora through non-probiotic methods.

Adverse effects and dropouts

Most of the studies did not report serious adverse events, and only four studies reported mild adverse effects such as dry mouth, senestopathia and diarrhoea. Schumann and colleagues²² reported two serious events related to major depressive episodes and deep leg vein thrombosis, but none of these events were determined to relate to the study interventions. In short, no matter which intervention was taken, the probability of serious adverse reactions was extremely low, and it is safe to improve the anxiety symptoms by regulating the intestinal flora. Besides, the dropout rates did have significant impacts on the results. The details of side effects and reasons for dropout are shown in [table 3](#).

DISCUSSION

Main findings

First of all, more than half of the 21 studies included in this paper showed that regulating intestinal flora can effectively improve anxiety symptoms. Of the 14 studies that used probiotics as the intervention, 36% of the studies were effective, while six of the seven studies

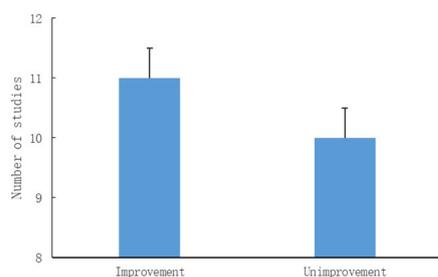


Figure 2 Outcomes of included studies. 11 of 21 studies showed that regulation of intestinal microbiota could improve anxiety symptoms, while 10 studies didn't show positive effects.

using non-probiotics as interventions were effective, and the effective rate was 86%. As for the five studies that used the TAU plus IRIF as interventions, only studies that conducted non-probiotic ways were positive; and non-probiotic interventions were also more effective in the studies that used IRIF alone, for 80% of studies could improve anxiety symptoms in the studies that performed non-probiotic interventions while 45% were effective in the studies that used probiotic ways. So we can easily find that although we can regulate the intestinal flora in two ways, the non-probiotic intervention is significantly better than the probiotic intervention. The reasons for this result may be as follows: (A) The energy source of gut microbiota growth is mainly food.²⁴ Adjusting the gut microbiota through modulating dietary structure can directly change the energy supplying structure of gut microbiota and this plays a decisive role in the growth of gut microbiota, so the effect is obvious. (B) Although the studies all conducted probiotic interventions, the species of the probiotics were diverse and there were survival competitions in implanted flora and primitive flora, which may lead to not all the imported probiotics being effectively implanted. (C) Most intervention times of included studies were 4–8 weeks. This might be too short to significantly increase the abundance of the imported microbiota, so that the subjects' original intestinal flora could not be effectively adjusted.

Second, 67% of the studies used probiotic intervention to regulate intestinal flora, while only 33% of the studies used non-probiotic ways such as low FODMAPS, scFOS and supplementary resistance dextrin. On the one hand, this indicates that more and more researchers have realised that microflora plays an increasingly important role in human health, but on the other hand, the function of diets in daily life has been neglected by people. As mentioned above, the effect of dietary structure adjustment is better than that of probiotic supplements. In the future, more attention can be paid to the regulation of intestinal flora through non-probiotic ways, or

the combination of probiotic and non-probiotic means, which may have unexpected effects.

In addition, the subjects were patients with chronic diseases comorbid with anxiety symptoms or healthy individuals. Chronic diseases included IBS, CFS, RA, obesity and fibromyalgia. Sixty-seven per cent of chronic disease subjects were patients with IBS, indicating that anxiety symptoms are common in intestinal-related chronic diseases. After reviewing the studies about the pathological mechanism of IBS published in recent years, Raskov and colleagues²⁵ found that the gut-brain axis played a central role in the persistence of IBS and the microbiota played a key role. In the study, the improvement of anxiety symptoms in patients with IBS by adjusting intestinal flora was further evidence of the gut-brain axis mechanism. Last but not least, the vast majority of studies have not reported serious adverse events related to interventions, regardless of what kinds of interventions were conducted. Another point that should be paid attention to was that 67% of six articles about healthy individuals have shown positive effects on anxiety symptoms, this may be strong evidence to support the hypothesis that anxiety symptoms can be relieved by modulating gut microbiota.

Only four studies reported mild adverse reactions such as dry mouth, internal perceptual discomfort and diarrhoea. In summary, more than half of the studies have shown that the intestinal flora could be modulated to alleviate anxiety symptoms and was extremely safe.

Limitations

Due to the differences in the research design types, subjects, interventions and anxiety assessment scales of the 21 articles included, the overall heterogeneity was too large and it was not suitable for meta-analysis. Fifty per cent of the 10 studies on IBS showed that the interventions were effective. Therefore, for patients with IBS, more studies are needed to verify whether it is possible to clinically treat the anxiety symptoms of patients with IBS by regulating intestinal flora. We did not register on PROSPERO whether the individuals had different kinds of diseases or were healthy individuals, rather we recorded all as having the same symptom—anxiety.

Implications

In the clinical treatment of anxiety symptoms, in addition to the use of psychiatric drugs for treatment, we can also consider regulating intestinal flora to alleviate anxiety symptoms. Especially for patients with somatic diseases who are not suitable for the application of psychiatric drugs for anxiety treatment, probiotic methods and/or non-probiotic ways like low FODMAPs can be applied flexibly according to clinical conditions. However, there are still some studies showing that the effect of regulating intestinal flora to improve anxiety symptoms is limited. Therefore, more relevant clinical intervention studies should be carried out with the unified anxiety assessment scales and statistical methods being used to clarify

the relationship between intestinal flora adjustment and improvement of anxiety symptoms.

Correction notice This article has been corrected since it was first published. This article was not published under an Open Access licence. This has now been corrected.

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