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Effect of regulating gut microbiota using probiotics on brain activity: protocol for a systematic review

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Title Page

Title

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Author's Contributions

LL developed the search strategy,TT and XL will search the databases and screen the eligibility of the retrieved studies. FL and XN will extract information from the eligible studies and prepare the information for data analysis. JC,MS and SZ will perform the data analysis. LL and LZ wrote the first draft of the protocol. In practice, LZ will monitor each procedure of the review and is responsible for quality control. All authors read the article and approved it for publication. All authors read and approved the final manuscript and order of authorship.

Conflict of Interest Disclosures

None reported.

Word Count

2391 words.

Key Words

Probiotics,gut microbiota,brain activity,fMRI

Effect of regulating gut microbiota using probiotics on brain activity: protocol for a systematic review

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Word Count:

2,391 words

Key Words:

probiotics,gut microbiota,brain activity,fMRI

Abstract

Introduction There is a growing number of randomized controlled trials (RCTs) that focus on brain activity changes detected by fMRI and gut microbiota composition changes after using probiotics.However,the effect of probiotics on brain activity through gut microbiota remains controversial in existing RCTs.Furthermore,to our knowledge,there is no systematic review to evaluate the effect of probiotics on brain activity through gut microbiota.Therefore,we aims to summarize literatures evaluating the potential association between probiotics,gut microbiota and brain activity to elucidate whether probiotics influence gut microbiota and affect brain activity through gut microbiota.

Methods and analysis CNKI,Wanfang Data,VIP Databases,SinoMed,PubMed,Web of Science,MEDLINE,EMBASE,the Cochrane Central Register of Controlled Trials, ClinicalTrials.gov and Cochrane Library CENTRAL were searched until July 2019. The Grey Literature in Europe (OpenSIGLE) database and Google search engine were also used.The reference lists of each included study were reviewed to determine whether there were any further relevant studies.RCTs using probiotics alone or with prebiotics (synbiotics) compared with a placebo/control will be included.We will use risk of bias assessment and the GRADE System to assess the quality of evidence.The results of the systematic review will be synthesized narratively in the domains of the three primary outcome measures:the changes in brain function and their association with the changes in clinical symptoms after probiotics' administration;the changes in composition or diversity of gut microbiota and their association with the changes in

clinical symptoms after probiotics' administration;the relationship between the functional alteration area of the brain and the changes in composition or diversity of gut microbiota after probiotics' administration.

Ethics and dissemination The results will be disseminated through a peer-reviewed publication. As no private and confidential patient data will be included in the reporting, there are no ethical considerations associated with this protocol.

PROSPERO registration number CRD42019145114

Article Summary

Strengths and limitations of this study

1. This is the first systematic review assessing the potential association between probiotics, gut microbiota and brain activity.

2. The study design adheres to all relevant guidelines for systematic reviews and meta-analysis.

3. The quality of evidence will be assessed by the GRADE system.

4. This systematic review will have inherent limitations related to the included studies such as risk of bias, methodological inconsistencies and incomplete outcome data.

5. We will only retrieve data from Chinese and English databases, which may limit available data or result in language bias.

Background

Probiotics are defined as "live microorganisms" that provide health benefits to the host when consumed in adequate amounts.[1] The use of probiotics has emerged as a principal approach to maintain the balance of the human gut microbiota.[2]

Probiotics have a strong ability to modulate the gut microbiota composition in healthy subjects,[3-4] leading to a significant reduction in several bacterial genera directly involved in the onset of gastrointestinal diseases.[3] Furthermore, probiotics regulate the gut microbiota of patients with gastrointestinal diseases and improve clinical symptoms.[5-6] These findings show that probiotics can be used to treat gastrointestinal

diseases by maintaining or changing the gut microbiota.

The gut microbiota includes bacteria, fungi, archaea, protozoa and viruses that interact with the host and each other to affect the host's physiology and health.[7] The microbiota has recently emerged as a key player in the gut-brain axis. This interaction between the brain and the microbiota has led to the recognition of a new term called 'microbiota-gut-brain axis'.[8] This interaction is bidirectional, meaning that disturbance in the complex community of microbiota (dysbiosis) can affect the brain and vice versa.[9] In clinical trials, neurological disorders were linked to gastrointestinal dysfunction and changes in gut microbiota.[10] *Candida albicans* exposure has been found to be associated with gastrointestinal dysfunction and impaired cognitive ability.[11] A meta-analysis that was performed on 13 studies up until 2016 has shown that there were significant differences in the levels of *Lactobacillus*, *Bifidobacterium* and *Faecalibacterium prausnitzii* in irritable bowel syndrome (IBS) patients compared with healthy controls.[12] Alteration in gut microbiota may contribute to IBS pathogenesis by altering the gut-brain axis.[13]

The underlying signaling mechanisms of these communicating networks between the gut flora and the gut-brain axis have been of special interest to researchers who are seeking potential therapeutic interventions.[9] The ingestion of probiotics by healthy humans showed diminished psychological symptoms, including anxiety symptoms,[14] while probiotic intervention significantly ameliorated the severity of depression in major depressive disorder patients,[15] and IBS patients.[16] The above results show that probiotics affect central nervous system diseases and this effect may be exerted through the microbiota-gut-brain axis. A study has systematically reviewed the effects of probiotics on central nervous system function in animals and humans suggesting that more research using both behavioral and neuroimaging measures on healthy volunteers and patients is needed in the future.[17] Furthermore, most of the psychological assessments in clinical trials were based solely on self-reported measures, hence firm conclusions regarding the effects of probiotics on the central nervous system cannot be drawn from these findings.

Given the difficulty of studying the brain at the cellular level in humans, neuroimaging has emerged as a tool for increasing our understanding of the microbiota-gut-brain axis. Advances in computational biology are beginning to

explain how these multifaceted and complex systems interact with each other.[18-19] Functional magnetic resonance imaging (fMRI) is able to measure moment-to-moment alterations in the blood oxygen content (the blood oxygen level dependent or BOLD signal).[20] Furthermore, it enables us to identify real-time changes in neurological activity and correlate these with changes in behavior or perception.[17,21-22] fMRI has been used successfully to identify differences in brain function in gastrointestinal disease states, such as IBS and inflammatory bowel disease, as well as in healthy people before and after chronic ingestion of probiotics.[20,23-24] Using fMRI, enables us to examine whether probiotics influence the gut microbiota and affect brain activity through the gut microbiota. It helps us better understand the microbiota-gut-brain axis, to develop a potential therapeutic method for central nervous system diseases in the future.

Recently, there were RCTs that focused on brain activity changes detected by fMRI and gut microbiota composition changes after using probiotic supplements. However, the effect of probiotics on brain activity through gut microbiota remains controversial in existing RCTs.[16,23,25] Furthermore, to our knowledge, there is no systematic review to evaluate the effect of probiotics on brain activity through gut microbiota. Therefore, we plan to systematically analyze all published RCTs in the medical literature to assess the effects of probiotics on the gut microbiota and brain activities. This will improve our understanding of the microbiota-gut-brain axis. Moreover, a deeper understanding of the underlying mechanisms will help refine the clinical use of probiotic supplements in the future.

Objectives

The objective of this systematic review is to summarize the literature evaluating the potential association between probiotics, gut microbiota and brain activity. fMRI of the participants after taking probiotics of any type, dosage, time point and duration will be evaluated. To this end, we aim to:

(1) Evaluate the relationship between the changes in clinical symptoms and the functional alteration area of the brain after probiotics' administration.

(2) Evaluate the relationship between the changes in clinical symptoms and the changes in composition or diversity of gut microbiota after probiotics' administration.

(3) Evaluate the relationship between the functional alteration area of the brain and the changes in composition or diversity of gut microbiota after probiotics' administration.

We aim to determine whether probiotics influence gut microbiota and affect brain activity through the gut microbiota.

Methods

Retrieval strategy

The following databases were searched until July 2019 for relevant RCTs: China National Knowledge Infrastructure (CNKI), Wanfang Data, VIP Databases, SinoMed, PubMed, Web of Science, MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials, ClinicalTrials.gov and Cochrane Library CENTRAL. The Grey Literature in Europe (OpenSIGLE) database and Google search engine were also searched. Furthermore, reference lists of each included study were reviewed to determine whether there were any further relevant studies. The search terms and search strategy are shown in Table 1:

Table 1. Search terms and search strategy

1.probiotic	16.fermented milk
2.culturelle	17.fMRI
3.Bifidobacteria	18.MRI
4.Lactobailli	19.MR
5.Acidophilus	20.functional magnetic resonance imaging
6.yogurt	21.magnetic resonance imaging
7.gut microbiota	22.neuroimaging
8.yeast	23.brain activity
9.Saccharomyces	24.MR tomography
10.prebiotic	25.NMR Imaging

11.Synbiotics	26.脑功能成像
12.Bacillus	27.核磁共振成像
13.enterococcus	28.功能性磁共振
14.streptococcus	29.益生菌
15.sour milk	30.益生元
	31.肠道菌群
32.“probiotic-related” terms	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16
33.“fMRI-related” terms	17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 24 OR 25
FINAL SEARCH TERMS	“32” AND “33”
FINAL SEARCH TERMS FOR CHINESE DATABASES	“29 OR 30 OR 31” AND “26 OR 27 OR 28”

Inclusion criteria

⑴ Type of study: clinical randomized controlled trials.

⑵ Participants: healthy individuals or patients.

⑶ Interventions: probiotics alone or together with prebiotics (synbiotics) compared with a placebo/control.

⑷ Outcomes: primary outcomes: brain imaging data obtained from fMRI and their association with clinical symptoms after using probiotics; changes in composition and diversity of the gut microbiota and their association with clinical symptoms after using probiotics; relationship between the functional alteration area of the brain and the changes in composition or diversity of the gut microbiota after probiotics’ administration. Secondary outcomes: adverse events and participants’ compliance during the trial.

Exclusion criteria

(1) Non-clinical randomized controlled trials.

(2) Editorials, literature reviews, and meta-analyses.

(3) Duplicate publications.

(4) Studies without full report of study results or primary data.

Outcome measures

The primary outcome measures for this review will be the assessment of brain activity changes, which were measured by fMRI, as they relate to gut microbiota regulated by probiotics.

Three different primary outcome measures will be obtained from the following primary research:

(1) The functional alteration area of the brain and its association with the changes in clinical symptoms after probiotics' administration.

(2) The changes in composition or diversity of the gut microbiota and their association with the changes in clinical symptoms after probiotics' administration.

(3) The relationship between the functional alteration area of the brain and the changes in composition or diversity of the gut microbiota after probiotics' administration.

Secondary outcome measures: the relevant adverse events and participants' compliance.

Study selection

All the studies retrieved through the search strategy will first be screened based on title and abstract using ENDNOTE, which can remove duplicates. Two reviewers will independently screen all titles and abstracts according to the inclusion criteria. Any disagreements will be resolved by discussion or a third reviewer. Full text of potentially eligible studies based on title and abstract will be screened for eligibility by two reviewers independently; disagreements will be resolved by discussion or a third reviewer if necessary. Reasons for study exclusions will be recorded.

Data extraction

Data will be extracted from each eligible study including the following information: authors; year of publication; journal; sample size; demographic characteristics of sample (age, ethnicity, sex); any information (e.g. type, duration, dose, time point) we can get on the intervention group and control/placebo group; data analysis strategy used; fMRI data analysis strategy used; outcomes (the functional alteration area of the brain and its association with the changes in clinical symptoms after probiotics' administration; gut microbiota composition or diversity changes and their association with the changes in clinical symptoms after probiotics' administration; relationship between the functional alteration area of the brain and the changes in composition or diversity of gut microbiota after probiotics' administration; adverse events and participants' compliance, and conclusions.

Quality assessment

Risk of bias assessment: the included studies will be assessed by two independent investigators using the Cochrane Risk of Bias Assessment Tool.[26] The assessment will include contents of random sequence generation, allocation concealment, blinding (of participants, personnel and outcome assessors), incomplete outcome data and selective outcome reporting and other sources of bias. The investigators' judgment will be categorized as "Low risk", "High risk" or "Unclear risk" of bias. The disagreements will be resolved by discussion or a third analyzer if necessary.

Quality of evidence assessment: two independent reviewers will assess overall quality of evidence using the Grading of Recommendations Assessment,Development and Evaluation(GRADE) system, examining the quality of the literature in five domains—risk of bias, publication bias, consistency, directness, and precision.[27] This system has four levels of classification ("high", "moderate", "low" and "very low") to which each study can be assigned based on different factors. Disagreements will be resolved by discussion or a third analyzer if necessary.

Strategy of data synthesis

The results of the systematic review will be synthesized narratively in the domains of the three primary outcome measures: the functional alteration area of the brain and its association with the changes in clinical symptoms after probiotics'

administration; the changes in composition or diversity of the gut microbiota and their association with the changes in clinical symptoms after probiotics' administration;

relationship between the functional alteration area of the brain and the changes in composition or diversity of the gut microbiota after probiotics' administration, and one secondary outcome measure: the relevant adverse events and participants' compliance. We will extract the functional alteration area of the brain to evaluate whether probiotics are effective in changing particular brain regions, where the altered regions in the brain are, and whether the altered brain regions were different between healthy individuals and patients. Additionally, we will evaluate the relationship between the changes in clinical symptoms and the functional alteration area of the brain after probiotics' administration. If possible from the number of studies available, we will use narrative review to explicate whether a "dose-dependent" or "type-dependent" relationship can be identified between probiotic supplements and brain activity changes. We will also evaluate the relationship between the changes in clinical symptoms and the changes in composition or diversity of the gut microbiota after probiotics' administration, and evaluate the relationship between the functional alteration area of the brain and the changes in composition or diversity of the gut microbiota after probiotics' administration. Finally, we will record all the adverse events and participants' compliance during the trial to assess the safety and popularization of probiotic supplements, which can be regarded as a new potential therapeutic method for central nervous system diseases in the future. To fully assess for gaps in evidence, we will do the risk of bias assessment, and the strength of evidence for this conclusion will be determined by the items evaluated in the GRADE framework described above. We will highlight areas that need further study and give suggestions for future studies.

Registration and reporting of results

We registered this study in PROSPERO international prospective register of systematic reviews on July 2019.[28] This protocol was drafted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMAP).[29] The methods and results of the systematic review will be reported in accordance with the PRISMAP guidelines.[30]

Patient and public involvement

No patients or public will be involved in this systematic review protocol.

Discussion

With the increasing annual growth in industrial production of food containing probiotics worldwide, the interest in elucidating how changes in the gut microbiota and changes in brain activity as a result of probiotics ingestion can serve as a new way for treating subjects with central nervous system diseases is increasing. In our systematic review, we will synthesize the evidence evaluating the association between probiotic supplements and brain activity changes and gut microbiota changes in humans to decipher whether probiotics influence brain activity and whether the effect of probiotics on brain activity is associated with changes in gut microbiota. This review provides a new way of thinking about the treatment of central nervous system diseases and health maintenance. To our knowledge, this will be the first systematic review focused on this topic.

Additionally, from the adverse events and participants' compliance with probiotic supplements, we can accumulate evidence for the popularization of probiotic therapy, which may be used to treat central system diseases in the future. This can help better understand probiotics' clinical application, effectiveness, and safety.

Furthermore, we will determine gaps or uncertainties in the existing literature, which requires further study. However, given the potential implications of such an association, this review will provide a solid foundation for the design and implementation of future studies that can better clarify the relationship between probiotics and gut microbiota and brain activities in humans.

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Author's Contributions

LL developed the search strategy,TT and XL will search the databases and screen the eligibility of the retrieved studies. FL and XN will extract information from the

eligible studies and prepare the information for data analysis. JC,MS and SZ will perform the data analysis. LL and LZ wrote the first draft of the protocol. In practice, LZ will monitor each procedure of the review and is responsible for quality control. All authors read the article and approved it for publication. All authors read and approved the final manuscript and order of authorship.

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Competing interests

None declared

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Table 1 Search terms and strategy

1.probiotic	16.fermented milk
2.culturelle	17.fMRI
3.Bifidobacteria	18.MRI
4.Lactobailli	19.MR
5.Acidophilus	20.functional magnetic resonance imaging
6.yogurt	21.magnetic resonance imaging
7.gut microbiota	22.neuroimaging
8.yeast	23.brian activity
9.Saccharomyces	24.MR tomography
10.prebiotic	25.NMR Imaging
11.Synbiotics	26.脑功能成像
12.Bacillus	27.核磁共振成像
13.enterococcus	28.功能性磁共振
14.streptococcus	29.益生菌
15.sour milk	30.益生元
	31.肠道菌群
32.“probiotic-related”terms	1 OR 2 OR 3 OR 4 OR 5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11 OR 12 OR 13 OR 14 OR 15 OR 16
33.“fMRI-related”terms	17 OR 18 OR 19 OR 20 OR 21 OR 22 OR 23 24 OR 25
FINAL SEARCH TERMS	“32” AND “33”
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Effect of regulating gut microbiota using probiotics on functional changes in the brain: protocol for a systematic review

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Abstract

Introduction There is a growing number of randomized controlled trials (RCTs) that focus on functional changes in the brain detected by functional magnetic resonance imaging(fMRI) and gut microbiota composition changes after using probiotics.

However,the effect of probiotics on functional changes in the brain through gut microbiota remains controversial in existing RCTs.Furthermore,to our knowledge,

there is no systematic review to evaluate the effect of probiotics on functional changes in the brain through gut microbiota.Therefore,we aim to summarize literatures evaluating the potential association between probiotics,gut microbiota and functional changes in the brain to elucidate whether probiotics influence gut microbiota and

affect functional changes in the brain through gut microbiota.

Methods and analysis CNKI,Wanfang Data,VIP Databases,SinoMed,PubMed,Web of Science,MEDLINE,EMBASE,Scopus,the Cochrane Central Register of Controlled Trials, ClinicalTrials.gov and Cochrane Library CENTRAL will be searched until July 2019. The Grey Literature in Europe (OpenSIGLE) database and Google search engine will also be used.The reference lists of each included study will be reviewed to determine whether there are any further relevant studies.RCTs using probiotics compared with a placebo/control will be included.We will use risk of bias assessment and the GRADE System to assess the quality of evidence.The results of the systematic review will be synthesized narratively in the domains of the three primary outcome measures:1) increased/decreased activity in brain regions or altered functional connectivity(FC) of brain detected by fMRI and their association with changes in behaviour,gastrointestinal/emotional symptoms after using probiotics;2) changes in composition and diversity of the gut microbiota and their association with changes in behaviour,gastrointestinal/emotional symptoms after using probiotics;3) increased/ decreased activity in brain regions or altered FC of brain detected by fMRI and the changes in composition or diversity of the gut microbiota after probiotics' administration.

Ethics and dissemination The results will be disseminated through a peer-reviewed publication. As no private and confidential patient data will be included in the reporting, there are no ethical considerations associated with this protocol.

PROSPERO registration number CRD42019145114

Article Summary

Strengths and limitations of this study

1. This is the first systematic review assessing the potential association between probiotics, gut microbiota and functional changes in the brain.

2. The study design adheres to all relevant guidelines for systematic reviews and meta-analyses.

3. The quality of evidence will be assessed by the GRADE system.

4. This systematic review will have inherent limitations related to the included studies such as risk of bias, methodological inconsistencies and incomplete outcome data.

5. We will only retrieve data from Chinese and English databases, which may limit available data or result in language bias.

Background

Probiotics are defined as "live microorganisms" that provide health benefits to the host when consumed in adequate amounts.[1] The use of probiotics has emerged as a principal approach to maintain the balance of the human gut microbiota.[2] Probiotics have a strong ability to modulate the gut microbiota composition in healthy subjects,[3-4] leading to a significant reduction in several bacterial genera directly involved in the onset of gastrointestinal diseases.[3] Furthermore, probiotics regulate the gut microbiota of patients with gastrointestinal diseases and improve clinical symptoms.[5-6] These findings show that probiotics can be used to treat gastrointestinal diseases by maintaining or changing the gut microbiota.

The gut microbiota includes bacteria, fungi, archaea, protozoa and viruses that interact with the host and each other to affect the host's physiology and health.[7] The microbiota has recently emerged as a key player in the gut-brain axis. This interaction between the brain and the microbiota in both animals and humans has led to the recognition of a new term called 'microbiota-gut-brain axis'.[8] This interaction is bidirectional, meaning that disturbance in the complex community of microbiota (dysbiosis) can affect the brain and vice versa.[9] A meta-analysis that was performed on 13 studies up until 2016 has shown that there were significant differences in the levels of *Lactobacillus, Bifidobacterium* and *Faecalibacterium prausnitzii* in irritable bowel syndrome (IBS) patients compared with healthy controls.[10] Alteration in gut microbiota may contribute to pathogenesis of IBS patients by altering the gut-brain axis.[11] Patients with neurological disorders were linked to gastrointestinal dysfunction and changes in gut microbiota.[12] And in another clinical trial, *Candida albicans* exposure has been found to be associated with gastrointestinal dysfunction and impaired cognitive ability.[13] The underlying signaling mechanisms of these communicating networks between the gut flora and the gut-brain axis have been of

special interest to researchers who are seeking potential therapeutic interventions.[9]

The ingestion of probiotics by healthy humans showed diminished psychological symptoms, including anxiety symptoms,[14] while probiotic intervention significantly ameliorated the severity of depression in major depressive disorder patients,[15] and IBS patients.[16] The above results show that gut microbiota is related to various diseases, and that modulating gut microbiota through probiotics may offer a potential treatment option. However, most of the psychological assessments in clinical trials[14,15,17] were based solely on self-reported measures, hence firm conclusions regarding the effects of probiotics cannot be drawn from these findings.

Given the difficulty of studying the brain at the cellular level in humans, neuroimaging has emerged as a tool for increasing our understanding of the brain. Advances in computational biology are beginning to explain how these multifaceted and complex systems interact with each other.[18-19] Neuroimaging tools as outcome measures are more objective than self-reported measures, which is an advantage over self-reported measures.fMRI is able to measure moment-to-moment alterations in the blood oxygen content (the blood oxygen level dependent or BOLD signal).[20] Furthermore,it enables us to identify real-time changes in neurological activity and correlate these with changes in behavior or perception.[21-23] fMRI has been used successfully to identify differences in brain function in gastrointestinal disease states, such as IBS and inflammatory bowel disease, as well as in healthy people before and after chronic ingestion of probiotics.[20,24-25] fMRI is effective predominantly because of its non-invasiveness,and it can accurately and intuitively observe the location and range of brain functional activity from the whole level under physiological condition.[26]Using task based fMRI or resting-state fMRI(rsfMRI),enables us to examine whether probiotics influence the gut microbiota and affect activity in brain regions or FC of brain through the gut microbiota. It helps us better understand the microbiota-gut-brain axis, to potentially help develop therapeutic methods for central nervous system diseases in the future.

Recently, there were RCTs that focused on increased/decreased activity in brain regions[24,27-28] or altered FC of brain[24,29] detected by task based fMRI or rs-fMRI after using probiotics in healthy subjects. However,some studies have found changes in gut microbiota,[27-28] behaviour,gastrointestinal/emotional symptoms,[27] while others have

not.[24] Furthermore, to our knowledge, there is no systematic review to evaluate the effect of probiotics on functional changes in the brain through gut microbiota. Therefore, we plan to systematically analyze all published RCTs in the medical literature to assess the effects of probiotics on the gut microbiota and functional changes in the brain. This will improve our understanding of the microbiota-gut-brain axis. Moreover, a deeper understanding of the underlying mechanisms will help refine the clinical use of probiotic supplements in the future.

Objectives

The objective of this systematic review is to summarize the literature evaluating the potential association between probiotics, gut microbiota and increased/decreased activity in brain regions or altered FC of brain in the healthy subjects after taking probiotics of any type, dosage, time point and duration will be evaluated. To this end, we aim to:

(1) Evaluate the relationship between the changes in behaviour,gastrointestinal/ emotional symptoms and increased/decreased activity in brain regions or altered FC of brain after probiotics' administration.

(2) Evaluate the relationship between the behaviour,gastrointestinal/emotional symptoms and the changes in composition or diversity of gut microbiota after probiotics' administration.

(3) Evaluate the relationship between increased/decreased activity in brain regions or altered FC of brain and the changes in composition or diversity of gut microbiota after probiotics' administration.

We aim to determine whether probiotics influence gut microbiota and affect functional changes in the brain through the gut microbiota.

Methods

Retrieval strategy

The following databases will be searched until July 2019 for relevant RCTs: China National Knowledge Infrastructure (CNKI), Wanfang Data, VIP Databases, SinoMed,

PubMed, Web of Science, MEDLINE, EMBASE, Scopus,the Cochrane Central Register of Controlled Trials, ClinicalTrials.gov and Cochrane Library CENTRAL. The Grey Literature in Europe (OpenSIGLE) database and Google search engine will also be searched. Furthermore, reference lists of each included study will be reviewed to determine whether there are any further relevant studies. The search terms will include probiotics (eg, “probiotics” or “culturelle” or “bifidobacteria” or “lactobailli” or “escherichia coli” or “yogurt” or “yeast” or “saccharomyces” or “bacillus” or “enterococcus” or “streptococcus” or “fermented milk” or “sour milk ” or “gastrointestinal microbiome” or “gut microbiota” or “gut microbiome” or “gut microflora” or “intestinal microflora” or “ intestinal bacteria” or “intestinal microbiome” or “microbiome” or “microbiota” or “bacteria” or “flora”), fMRI(eg, “magnetic resonance imaging” or “fMRI” or “functional magnetic resonance imaging” or “neuroimaging” or “MRI” or “brian activity” or “MR tomography” or “NMR Imaging” or “MR” or “independent component analysis” or “ functional network connectivity” or “amplitude of low frequency fluctuations” or “regional homogeneity” or “functional connectivity” or “central” or “brain” or “neuro” or “ALFF” or “FALFF” or “REHO” or “ROI ” or “FC” or “ICA”).The following terms will be used in the Chinese database searches:“Yi sheng jun”, “Chang dao jun qun”, “Jun qun”, “Nao gong neng cheng xiang”, “Gong neng xing ci gong zhen”, “He ci gong zhen cheng xiang” and “He ci”.The search strategy for the PubMed database is shown in Table 1(in the supplementary file),this strategy will be modified appropriately for other databases.

Inclusion criteria

(1) Type of study: randomized clinical controlled trials.

(2) Participants: healthy individuals

(3) Interventions: probiotics compared with a placebo/control.

(4) Outcomes measures:the primary outcome measures for this review will be the assessment of functional changes in the brain,which were measured by fMRI, as they relate to gut microbiota regulated by probiotics.Three different primary outcome measures will be obtained from the following primary research:1) increased/decreased activity in brain regions or altered FC of brain detected by fMRI and their association

with changes in behaviour,gastrointestinal/emotional symptoms after using probiotics;

2) changes in composition and diversity of the gut microbiota and their association with changes in behaviour,gastrointestinal/emotional symptoms after using probiotics;

3) increased/decreased activity in brain regions or altered FC of brain detected by fMRI and the changes in composition or diversity of the gut microbiota after probiotics' administration.Secondary outcomes: adverse events and participants' compliance during the trial.

Exclusion criteria

(1) Non-randomized clinical controlled trials.

(2) Editorials, literature reviews, and meta-analyses.

(3) Duplicate publications.

(4) Studies without full report of study results or primary data.

Study selection

All the studies retrieved through the search strategy will first be screened based on title and abstract using ENDNOTE, which can remove duplicates. Two reviewers will independently screen all titles and abstracts according to the inclusion criteria. Any disagreements will be resolved by discussion or a third reviewer. Full text of potentially eligible studies based on title and abstract will be screened for eligibility by two reviewers independently; disagreements will be resolved by discussion or a third reviewer if necessary. Reasons for study exclusions will be recorded.

Data extraction

Data will be extracted from each eligible study including the following information: authors; year of publication; journal; sample size; demographic characteristics of sample (age, ethnicity, sex); any information (e.g. type, duration, dose, time point) we can get on the intervention group and control/placebo group; data analysis strategy used;fMRI data analysis strategy used; outcomes,and conclusions.

Quality assessment

Risk of bias assessment: the included studies will be assessed by two independent investigators using the Cochrane Risk of Bias Assessment Tool.[30] The assessment will include contents of random sequence generation, allocation concealment, blinding (of participants, personnel and outcome assessors), incomplete outcome data and selective outcome reporting and other sources of bias. The investigators' judgment will be categorized as "Low risk", "High risk" or "Unclear risk" of bias. The disagreements will be resolved by discussion or a third analyzer if necessary.

Quality of evidence assessment: two independent reviewers will assess overall quality of evidence using the Grading of Recommendations Assessment,Development and Evaluation(GRADE) system, examining the quality of the literature in five domains—risk of bias, publication bias, consistency, directness, and precision.[31] This system has four levels of classification ("high", "moderate", "low" and "very low") to which each study can be assigned based on different factors. Disagreements will be resolved by discussion or a third analyzer if necessary.

Strategy of data synthesis

The results of the systematic review will be synthesized narratively in the domains of the three primary outcome measures:1) increased/decreased activity in brain regions or altered FC of brain and their association with changes in behaviour,gastrointestinal/ emotional symptoms after using probiotics;2) changes in composition and diversity of the gut microbiota and their association with changes in behaviour,gastrointestinal /emotional symptoms after using probiotics;3) increased/decreased activity in brain regions or altered FC of brain and the changes in composition or diversity of the gut microbiota after probiotics' administration.and one secondary outcome measure: the relevant adverse events and participants' compliance. We will extract the increased/ decreased activity in brain regions or altered FC of brain to evaluate whether probiotics are effective in activating/deactivating particular brain regions or are effective in altering FC of brain, where the functional changes in the brain are. Additionally, we will evaluate the relationship between the changes in behaviour, gastrointestinal/emotional symptoms and the increased/decreased activity in brain

regions or altered FC of brain after probiotics' administration. If possible from the number of studies available, we will use narrative review to explicate whether a "dose-dependent" or "type-dependent" relationship can be identified between probiotic supplements and functional changes in the brain. We will also evaluate the relationship between the changes in behaviour,gastrointestinal/emotional symptoms and the changes in composition or diversity of the gut microbiota after probiotics' administration, and evaluate the relationship between the increased/decreased activity in brain regions or altered FC of brain and the changes in composition or diversity of the gut microbiota after probiotics' administration. Finally, we will record all the adverse events and participants' compliance during the trial to assess the safety and popularization of probiotic supplements, which can be regarded as a new potential therapeutic method for central nervous system diseases in the future. To fully assess for gaps in evidence, we will do the risk of bias assessment, and the strength of evidence for this conclusion will be determined by the items evaluated in the GRADE framework described above. We will highlight areas that need further study and give suggestions for future studies.

Registration and reporting of results

We registered this study in PROSPERO international prospective register of systematic reviews on July 2019.[32] This protocol was drafted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMAP).[33] The methods and results of the systematic review will be reported in accordance with the PRISMAP guidelines.[34]

Patient and public involvement

No patients or public will be involved in this systematic review protocol.

Discussion

According to the Global Burden of Disease Study, 322 and 264 million people worldwide suffered from depression and anxiety, respectively in 2015. This is an increase of 18.4% and 14.9% over the 2005 figures.[35] There were more than 80 million stroke survivors in the world,[36] 43.8 million people with dementia,[37] 45.9 million patients with an active epilepsy,[38] and 6.1 million individuals with Parkinson's

disease.[39] Globally, in 2016, neurological disorders were the leading cause of disability (276 million disability-adjusted life-years) and the second leading cause of deaths (9 million) in the world.[40] With the increasing annual growth in industrial production of food containing probiotics worldwide, the interest in elucidating how changes in the gut microbiota and functional changes in brain as a result of probiotics ingestion can serve as a new way for treating subjects with central nervous system diseases is increasing. In our systematic review, we will synthesize the evidence evaluating the association between probiotic supplements and functional changes in the brain and gut microbiota changes in humans to decipher whether probiotics influence functional changes in the brain and whether the effect of probiotics on functional changes in the brain is associated with changes in gut microbiota. This review provides a new way of thinking about the treatment of central nervous system diseases and health maintenance. To our knowledge, this will be the first systematic review focused on this topic.

Additionally, from the adverse events and participants' compliance with probiotic supplements, we can accumulate evidence for the popularization of probiotic therapy, which may be used to treat central system diseases in the future. This can help better understand probiotics' clinical application, effectiveness, and safety.

Furthermore, we will determine gaps or uncertainties in the existing literature, which requires further study. However, given the potential implications of such an association, this review will provide a solid foundation for the design and implementation of future studies that can better clarify the relationship between probiotics and functional changes in the brain and gut microbiota and in humans.

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Author's Contributions

LL developed the search strategy,TT and XL will search the databases and screen the eligibility of the retrieved studies. FL and XN will extract information from the eligible studies and prepare the information for data analysis. JC,MS and SZ will

perform the data analysis. LL and LZ wrote the first draft of the protocol. In practice, LZ will monitor each procedure of the review and is responsible for quality control. All authors read the article and approved it for publication. All authors read and approved the final manuscript and order of authorship.

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Competing interests

None declared

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Search strategy for the Pubmed database is as follows,and filters in article types,publication dates and species,we chose clinical trails,10years and humans respectively.

Table 1 Search strategy for the Pubmed database

1.probiotics. Mesh.	27.magnetic resonance imaging. Mesh.
2.culturelle. ti, ab	28.fMRI. ti, ab
3.bifidobacteria. ti, ab	29.MRI. ti, ab
4.lactobailli. ti, ab	30.MR. ti, ab
5.escherichia coli. ti, ab	31.functional magnetic resonance imaging. ti, ab
6.yogurt. ti, ab	32.NMR Imaging. ti, ab
7.yeast. ti, ab	33.neuroimaging. ti, ab
8.saccharomyces. ti, ab	34.brian activity. ti, ab
9.bacillus. ti, ab	35.MR tomography. ti, ab
10.enterococcus. ti, ab	36.independent component analysis. ti, ab
11.streptococcus. ti, ab	37. functional network connectivity. ti, ab
12.fermented milk. ti, ab	38.amplitude of low frequency fluctuations. ti, ab
13.sour milk. ti, ab	39.regional homogeneity. ti, ab
14. 1 or 2-13	40.functional connectivity. ti, ab
15.gastrointestinal microbiome. Mesh.	41.central. ti, ab
16.gut microbiota. ti, ab	42.brain. ti, ab
17.gut microbiome. ti, ab	43.neuro. ti, ab
18.gut microflora. ti, ab	44.ALFF. ti, ab
19.intestinal microflora. ti, ab	45.FALFF. ti, ab
20.intestinal bacteria. ti, ab	46.ReHo. ti, ab
21.intestinal microbiome. ti, ab	47.ROI. ti, ab
22.microbiome. ti, ab	48.ICA. ti, ab
23.microbiota. ti, ab	49.FC. ti, ab
24.flora. ti, ab	50. 27 or 28-49
25.bacteria. ti, ab	51. 14 or 26
26. 15 or 16-25	52.(Final search) 50 and 51