Effects of the Human Gut Microbiota on Cognitive Performance, Brain Structure and Function: A Narrative Review

by Katie Louise Tooley ✉

Cognition & Behaviour, Land Division (Edinburgh), Defence Science & Technology, Department of Defence, Edinburgh, SA 5111, Australia

nutrients

Table 3. Human studies describing interventions on the gut microbiota and the effects on cognition, brain structures and function.

Author/Year	Participants/Sample (± SD)	Sex (M/F)	Study Design	Treatment	Dose/Frequency	Assessment	Main Findings— Microbiome Link
Probiotics							
Allen et al. (2016) [22]	$n = 22$ healthy males (22.5 ± 1.2 SEM y)	22/0	Repeated measures, placebo-controlled within-subject (blinding not stated)	*Bifidobacterium longum* 1714 strain	PRO = 1 × 10^9 cfu/stick or PLA; 1 stick/day 4 weeks each. PLA→PRO	Cognitive tasks: CANTAB done with EEG	mild improvement vs. PLA in visuospatial memory; EEG profile consistent with improved memory
Kelly et al. (2017) [33]	Placebo-Probiotic group $n = 15$ (23.6 ± 1.0 year); Probiotic-Placebo group $n = 14$ (25.6 ± 1.1 year)	29/0	Randomised Placebo-controlled cross-over design (wash-out and randomisation not detailed)	*Lactobacillus rhamnosus* (JB-1)	Active treatment contained 1 × 10^9 cfu/capsule; f = 1 daily 4 wk then cross-over	CANTAB	No improvement in cognitive parameters
Lew et al. (2018) [7]	Moderately stressed adults: $n = 51/66$ PLA (32.1 ± 11.4 year); $n = 52/66$ probiotic (31.3 ± 10.8 year)	12/39 12/40 (24/79)	RDBPC	*Lactobacillus plantarum* P8 (isolated from traditionally fermented sour milk —Mongolia)	2 g sachet of probiotic P8 or PLA P8 dose: 2 × 10^{10} cfu/day Daily 12 weeks	CogState Brief Battery	Social emotional speed response and verbal & memory learning improved; Cognitive and memory traits correlated with stress and anxiety. Sex different responses.
Tillisch et al. (2013) [8]	Females aged females (22.8 ± 2.7 year); $n = 12$ in fermented probiotic group, $n = 11$ in non-fermented control; $n = 13$ nil intervention	0/36	RDBPC (treatment, PLA and nil intervention)	Fermented milk containing *Bifidobacterium animalis* subsp *lactis* (strain number I-2494, *Streptococcus themophilus* and *Lactobacillus bulgaricus* (Danone Research Facilities)	*lactis* = 1.25 × 10^{10}, *thermophilus* + *bulgaricus* = 1.2 × 10^9; cfu/cup; f = daily 4 weeks	fMRI	affected activity of brain areas controlling central processing (emotion & sensation)

We can see a number of "classic" probiotic interventions attempting to modulate cognitive function via the microbiota gut-brain axis.

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Study	Sample		Design	Intervention	Dose	Measures	Outcomes
Bagga et al. (2018) [6]	Healthy volunteers: $n = 15$ no-intervention control (26.9 ± 5.0 year); $n = 15$ PLA (27.3 ± 5.8 year); $n = 15$ probiotic (28.3 ± 4.2 year)	7/8 9/6 7/8 (22/23)	RDBPC (randomisation and blinding not specified)	9 strains: Lactobacillus casei W56, L. acidophilus W22, L. paracasei W20, Bifidobacterium lactis W51, L. salivarius W24, Lactococcus lactis W19, B. lactis W52, L. plantarum W62 and B. bifidum W23	7.5×10^9/3 g dose (see extra table information) vs. PLA or CON; f = daily 4 weeks	PANAS; SCL-90; ADS; LEIDS; fMRI with emotional decision making and recognition tasks	Microbiome composition mirrored self-reported behavioural measures and memory performance; potential link between specific Bacteroides, brain memory and recognition
Bagga et al. (2019) [34] - Epub May 2018	Healthy volunteers: $n = 15$ no-intervention control (26.9 ± 5.0 year); $n = 15$ PLA (27.3 ± 5.8 year); $n = 15$ probiotic (28.3 ± 4.2 year)	7/8 9/6 7/8 (22/23)	RDBPC (randomisation and blinding not specified)	See Bagga 2018 study	7.5×10^9/3 g dose vs. PLA or CON; f = daily 4 weeks	fMRI	Changes in functional connectivity (link to depression and stress disorders) vs. PLA and CON
Roman et al. (2018) [35]	$n = 40$ fibromyalgia patients; complete study: probiotic $n = 16/20$ (55.0 ± 2.1 year); PLA $n = 15/20$ (50.3 ± 2.0 year)	1/15 2/13 (3/28)	Pilot RDBPC (blinding not specified)	ERGYPHILUS Plus (Laboratorios NUTERGIA, Spain): Lactobacillus Rhamnosus GG, Lactobacillus Casei, Lactobacillus Acidophilus, Bifidobacterium Bifidus.	6×10^9/capsule (See Footnote) 2 capsules, twice daily; 8 weeks	Two-choice task and Iowa gambling task (impulsive choice and decision-making); mini mental state examination; urinary cortisol	probiotics improved impulsivity and decision-making in fibromyalgia patients
Prebiotics							
Schmidt et al. (2015) [23]	$n = 15$ PLA (23.3 ± 3.9 year); $n = 15$ FOS (24.5 ± 3.9 year); $n = 15$ B-GOS (23.3 ± 4.0 year)	7/8 8/7 7/8 (22/23)	RDBPC	Fructooligosaccharides (FOS) or Bimuno®-galacto-oligosaccharides (B-GOS)	5.5 g of FOS, B-GOS or PLA; Daily; 3 weeks	Attentional dot-probe task	B-GOS increased attentional vigilance to positive to negative stimuli
Smith et al. (2015) [36]	$n = 47$ (ave 23.0 years, range 19–30 years)	19/28	Cross-over (randomisation or blinding not detailed)	Oligofructose-Enriched Inulin or PLA added to de-caffeinated tea or de-caffeinated coffee	Pre-fasted 5 g prebiotic f = once0–4 h (acute effects)	Memory tasks; psychomotor tasks (simple reaction and selective attention tasks); sustained attention	Episodic memory tasks improved Psychomotor performance and selective attention unchanged.

But we also see a number of novel interventions based on prebiotic and complex saccharides, known to feed Bifidobacteria.

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Paraprobiotics							
Chung et al. (2014) [37]	Healthy adults *n* = 36/39: *n* = 10 PLA (64.5 ± 4.8 year); *n* = 10,500 mg (64.5 ± 2.2 year); *n* = 71,000 mg (64.43 ± 4.5 year); *n* = 92,000 mg (66.6 ± 5.0 year)	4/6 9/1 2/5 5/4 (20/16)	RDBPC (blinding not specified)	*Lactobacillus helveticus* (IDCC3801) Fermented (heat-treated) milk (LHFM); supernatant extracted and placed in tablet form.	took 4 tablets daily to reach a conc. of 500, 1000, 2000 or 0 mg (PLA) 12 weeks	Digit-span; Story recall; verbal learning; RVIP (cognitive fatigue measure); stroop; serial 3 s and 7 s	minor improvement in RVIP accuracy only for low dose of heat-treated fermented milk tablet
Ohsawa et al. (2018) [38]	All with mild memory deficits: *n* = 31/31 in fermented probiotic milk (58.5 ± 6.5 year); *n* = 29/30 PLA (57.8 ± 5.9 year)	13/18 13/16 (26/34)	RDBPC (blinding not specified)	Lactobacillus helveticus-fermented milk containing 2.4 mg lactononadeca-peptide (NIPPLTQTPV VVPPFLQPE). PLA contained no active ingredient	190 g drink with/without fermented peptide (2.4 mg) One daily 8 weeks	RBANS	Improvement in total RBANs and delayed memory score. Attention and coding score also improved. All other measures NS
Synbiotics							
Tooley et al. (2018) [39] - Conf abstract (manuscript in preparation)	Healthy young University Students: *n* = 34 Synbiotic; *n* = 33 PLA	16/51	RDBPC	*Lactobacillus acidophilus* L10 and *Bifidobacterium lactis* B94 plus arabinogalactan, inulin and trehalose	1.5×10^{10} of both bacteria strains cfu/5 g dose f = daily 4 weeks	Cognitive Battery	Synbiotic improved memory: immediate & delayed recall. Vigilance, attention, simple reaction time, executive control NS.

Along with some interesting paraprobiotics and synbiotics.

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Connecting science, microbes and health

Fiber

✓ Not digested by humans, but some fibers are utilized by gut microbes

✓ Naturally present in many whole grains, fruits, vegetables and legumes

✓ Adequate Intake values specified. Daily Value of 28 g/d based on 2000 kcal/d diet

✓ Can be soluble or insoluble

Prebiotics

✓ Not digested by humans, but acted on by gut microbes

✓ Naturally present in a wide range of foods from plants (e.g. chicory root , vegetables, whole grains). Usually isolated from whole plants or synthesized from sugars

✓ No Adequate Intake level or Daily Value

✓ Many current prebiotics are a type of soluble dietary fiber

Insoluble	Soluble	Fiber prebiotics	Non-fiber prebiotics
e.g. Cellulose	e.g. Psyllium	Inulin, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS). Promising candidates are resistant starch, polydextrose, xylo-oligosaccharide (XOS) and isomalto-oligosaccharide (IMO).	Lactulose, promising candidates Polyphenolics, and polyunsaturated fatty acids

Some microbiome modulation

Proven microbiome modulation associated with health benefits

Degree of microbiome modulation

Broccoli has 4 g fiber

What is a prebiotic?

In simple terms, a prebiotic is food for beneficial members of your resident microbial community – we can't digest prebiotics, but certain beneficial microbes can. Your resident microbes can produce a variety of beneficial compounds (for example, short chain fatty acids) from utilization of prebiotics. These can promote a healthy gut – and beyond. In more technical terms, a prebiotic is a substance that is selectively utilized by host microorganisms conferring a health benefit.

What is fiber?

Fibers are non-digestible plant-derived carbohydrates comprising at least 3 units of individual sugars. Most fibers are components of plants. Depending on regulations where you live, if fiber is isolated from whole plants or synthesized from sugars, demonstration of physiological benefits is needed to be able to call them 'fiber' on a food label.

Black beans have 15 g fiber per cup

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Synbiotic was a term originally proposed in 1995 to refer to a combination of a probiotic and a prebiotic. In 2019, a group of scientists met to discuss specifics of this class of substances and to propose a new definition.

Synbiotics

A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms* that confers a health benefit on the host.**

Initially, the idea of synbiotics was to add a probiotic and a prebiotic together. This approach would require that each component meet the criteria for either probiotic or prebiotic.
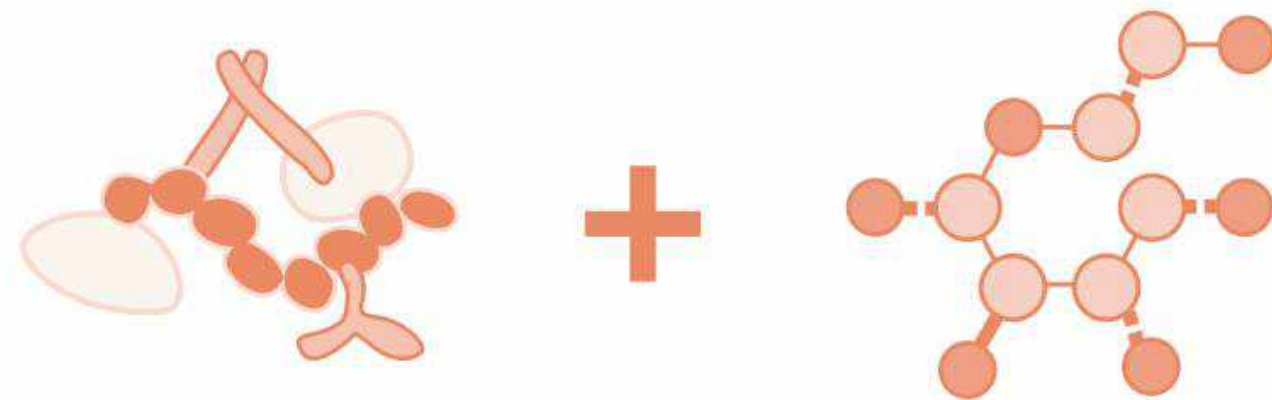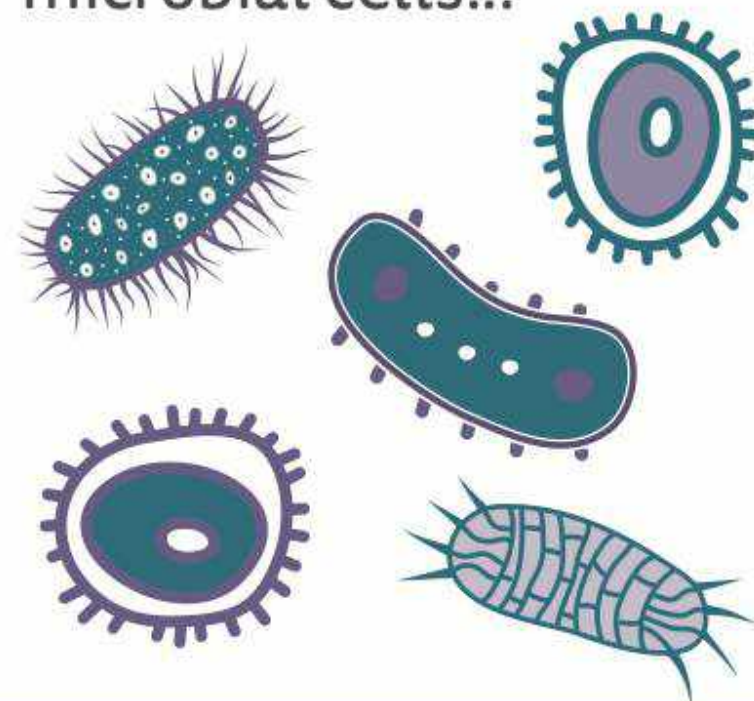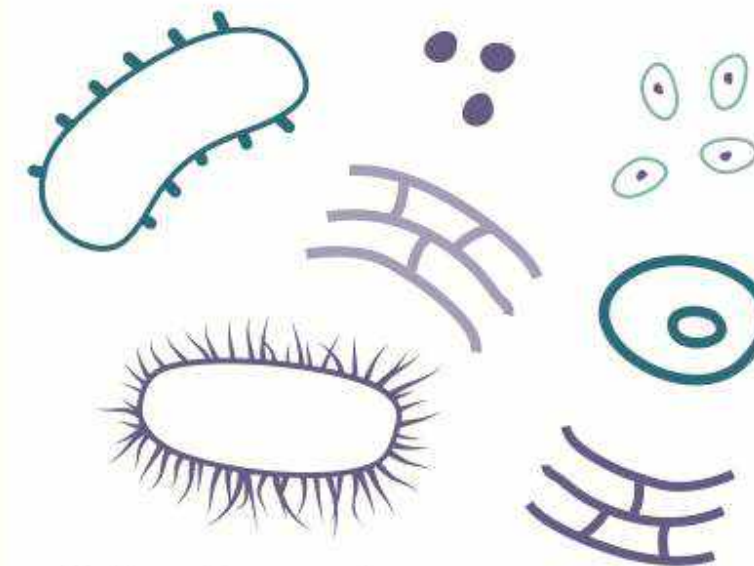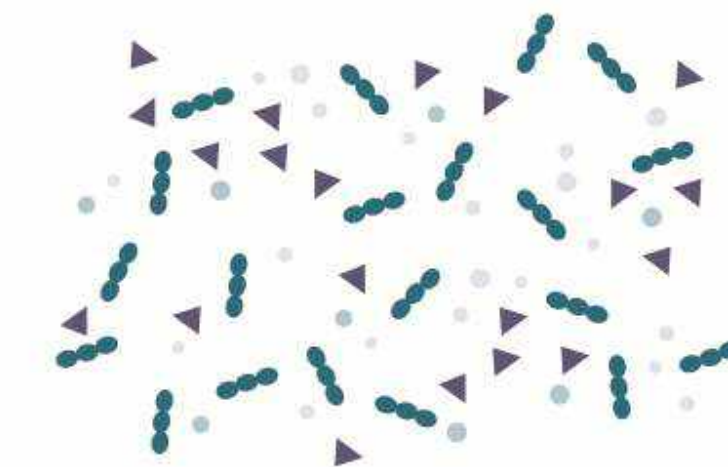
When defining synbiotics, scientists wanted to be sure that innovative products could use this designation. They realized that it would be possible to design a combination of a live microbe and a prebiotic-like substance that could work together – the substance feeding the live microbe – but neither on its own would necessarily meet the definitions of "probiotic" and "prebiotic" (dose and evidence of health benefit). **Hence the definition is not simply a probiotic + prebiotic**.

Patient Reported Outcome Measures

PROs or PROMs?

Patient-**r**eported **o**utcomes are referred to as **PROs,**

whist **patient**-**r**eported **o**utcome **m**easures are referred to as PROMs.

Both acronyms are used routinely in clinical literature.

I've selected the following scales as my personal favourites, with a rationale provided in the upcoming slides.

The Perceived Stress Scale

Item	never	almost never	sometimes	fairly often	very often
In the last month, how often have you been upset because of something that happened unexpectedly?	○	○	○	○	○
In the last month, how often have you felt that you were unable to control the important things in your life?	○	○	○	○	○
In the last month, how often have you felt nervous and "stressed"?	○	○	○	○	○
In the last month, how often have you dealt successfully with irritating life hassles?	○	○	○	○	○
In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?	○	○	○	○	○
In the last month, how often have you felt confident about your ability to handle your personal problems?	○	○	○	○	○
In the last month, how often have you felt that things were going your way?	○	○	○	○	○
In the last month, how often have you found that you could not cope with all the things that you had to do?	○	○	○	○	○
In the last month, how often have you been able to control irritations in your life?	○	○	○	○	○
In the last month, how often have you felt that you were on top of things?	○	○	○	○	○
In the last month, how often have you been angered because of things that happened that were outside of your control?	○	○	○	○	○
In the last month, how often have you found yourself thinking about things that you have to accomplish?	○	○	○	○	○
In the last month, how often have you been able to control the way you spend your time?	○	○	○	○	○
In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	○	○	○	○	○

- **The Perceived Stress Scale (PSS) is one of the most highly cited scales for the measurement of the degree to which life appears stressful.**

- **As of 1st December 2021, the original paper entitled "A global measure of perceived stress" by Sheldon and colleagues, published in the Journal of Health and Social Behaviour in 1983, has been cited 27,361 times.**

PSS examples

Brain, Behavior, & Immunity - Health
Volume 2, February 2020, 100029

Acute intake of *B. longum* probiotic does not reduce stress, anxiety, or depression in young adults: A pilot study

Michael P. Siegel [a, b], Sarah M. Conklin Ph.D. [a]

The Perceived Stress Scale (PSS) was used to assess perceived stress. The PSS is a widely used, 10-item, questionnaire that measures the degree to which a participant's life is perceived as stressful (Cohen and Williamson, 1988). Participants were asked to report the degree to which they have felt a certain way, with responses ranging from *never* (0) to *very often* (4) (Cohen and Williamson, 1988; Deckro et al., 2002). Recent data showed that an average PSS score among an 18–29 year old cohort (N = 645) was 14.2(6.2) (Cohen and Williamson, 1988).

The Perceived Stress Scale is used by eminent scientists in the gut-brain axis research.

Brain, Behavior, & Immunity - Health
Volume 10, January 2021, 100174
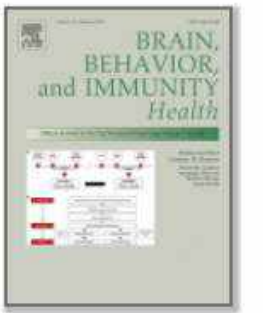
Full Length Article

Improvements in sleep indices during exam stress due to consumption of a *Bifidobacterium longum*

Gerard M. Moloney [a, b, 2], Caitriona M. Long-Smith [a, 2], Amy Murphy [a, c, d], Danielle Dorland [a], Sara Firuzeh Hojabri [a], Loreto Olavarría Ramirez [a], David Campos Marin [a], Thomaz F.S. Bastiaanssen [a], Anne-Marie Cusack [a], Kirsten Berding [a], Fiona Fouhy [a, c], Andrew P. Allen [a], Catherine Stanton [a, c, d], Gerard Clarke [a, d], Timothy G. Dinan [a, d, 1], John F. Cryan [a, b, 1]

Participants filled in self-report scales and questionnaires, including the Food Frequency Questionnaire (FFQ), International Physical Activity Questionnaire (IPAQ), Gastrointestinal Visual Analogue Scale (GI-VAS), Bristol Stool Chart, Pittsburgh Sleep Quality Index (PSQI), Perceived Stress Scale (PSS), Reading the Mind in the Eyes, and the Beck's Depression Inventory second edition (BDI-II). Cognitive performance was measured using a battery of tests from the CANTAB suite. At the post-intervention visit, the Primary Appraisal Secondary Appraisal (PASA) was additionally included.

Why the Perceived Stress Scale

- **The PSS provides a useful measure of global stress levels, i.e. it has been scientifically validated to cover a wide range of sources of stress, e.g. financial, emotional, etc.**

- **The scale goes from 0 to 56, with 0 being the most resilient, and scores over 28 meaning high susceptibility to suffering from the negative effects of stress.**

Measuring Anxiety

Original Investigation

JAMA Internal Medicine

May 22, 2006

A Brief Measure for Assessing Generalized Anxiety Disorder
The GAD-7

Robert L. Spitzer, MD; Kurt Kroenke, MD; Janet B. W. Williams, DSW; et al

The GAD-7 is a short test that checks symptoms and can be used assess the severity of generalised anxiety.

Over the last 2 weeks, how often have you been bothered by the following problems?

Item	not at all	several days	more than half the days	nearly every day
Feeling nervous, anxious or on edge	○	○	○	○
Not being able to stop or control worrying	○	○	○	○
Worrying too much about different things	○	○	○	○
Trouble relaxing	○	○	○	○
Being so restless that it is hard to sit still	○	○	○	○
Becoming easily annoyed or irritable	○	○	○	○
Feeling afraid as if something awful might happen	○	○	○	○

More about the GAD-7

- The possible scores on the GAD-7 run from 0 to 21. In the study by Spitzer et al. (2006), more than 2000 patients in primary care settings participated.

- Of the patients who were known to suffer from generalised anxiety disorder, the average GAD-7 score was 14.4.

- Of the patients that were known not to suffer from the disorder, the GAD-7 score was 4.9.
- According to the authors, people with a score of 10 or greater might suffer from generalised anxiety disorder.

- Women suffer considerably more from generalised anxiety disorder than men.

Altered gut microbiota profile in patients with generalized anxiety disorder

Hai-yin Jiang [a, 1], Xue Zhang [a, 1], Zheng-he Yu [b], Zhe Zhang [c], Min Deng [a], Jian-hua Zhao [d], Bing Ruan [a] ✉

Abstract

Close relationships have recently been established between gut microbiota and some mental disorders. Here, we performed a systematic comparative analysis of the gut microbiome in patients with generalized anxiety disorder (GAD) and healthy controls (HCs). We first conducted a cross-sectional study of 40 patients with GAD in the active state and 36 HCs. Second, subgroup analysis consisting of 12 antidepressant-naive patients and 22 controls was performed to validate the results. Finally, a prospective study was performed in a subgroup of nine patients with GAD who underwent analysis in the active state of anxiety and in remission. Compared with the HCs, we found markedly decreased microbial richness and diversity, distinct metagenomic composition with reduced short-chain fatty acid (SCFA)-producing bacteria (associated with a healthy status) and overgrowth of bacteria, such as *Escherichia-Shigella*, *Fusobacterium* and *Ruminococcus* gnavus. Unexpectedly, these changes in the genera were not reversed in remissive GAD. This study identified microbiota dysbiosis of gut microbiota in GAD patients, suggesting that targeting the microbiome may be a useful therapeutic and preventive target for GAD.

Why the GAD-7?

The GAD-7 is easy to administer and to score, and can be used both in paper and electronically.

Measuring Cognition

An Investigation Into Physical Frailty as a Link Between the Gut Microbiome and Cognitive Health

Serena Verdi,[1,2] Matthew A. Jackson,[1,3] Michelle Beaumont,[1] Ruth C. E. Bowyer,[1] Jordana T. Bell,[1] Tim D. Spector,[1] and Claire J. Steves[1,4,*]

Cognitive Measures

To acknowledge the complexity and variation that occurs with cognitive traits, we used four different clinically validated measures of cognitive function: verbal Fluency Test, Deary-Liewald Reaction Time Test (DLRT) and Mini Mental State Examination (MMSE) and Cambridge Neuropsychological Test Automated Battery-Paired-Associated Learning Test (CANTAB-PAL). These cognitive data constitute all the cognitive measures collected during the routine TwinsUK cohort clinical visits between 2013 and 2016 and were matched to the nearest collected fecal sample.

There are too many cognitive measures to be covered in detail in this masterclass. My choice is the CANTAB Cognitive Assessment Suite because of its availability as an online suite that's easy to administer and it's automatically scored.

It is however quite tricky to understand unless properly trained, and translating the findings isn't always straightforward.

What about gut-brain axis biomarkers?

The following slides illustrate some of the key biomarkers featured in gut-brain axis clinical trials alongside microbial sequencing.
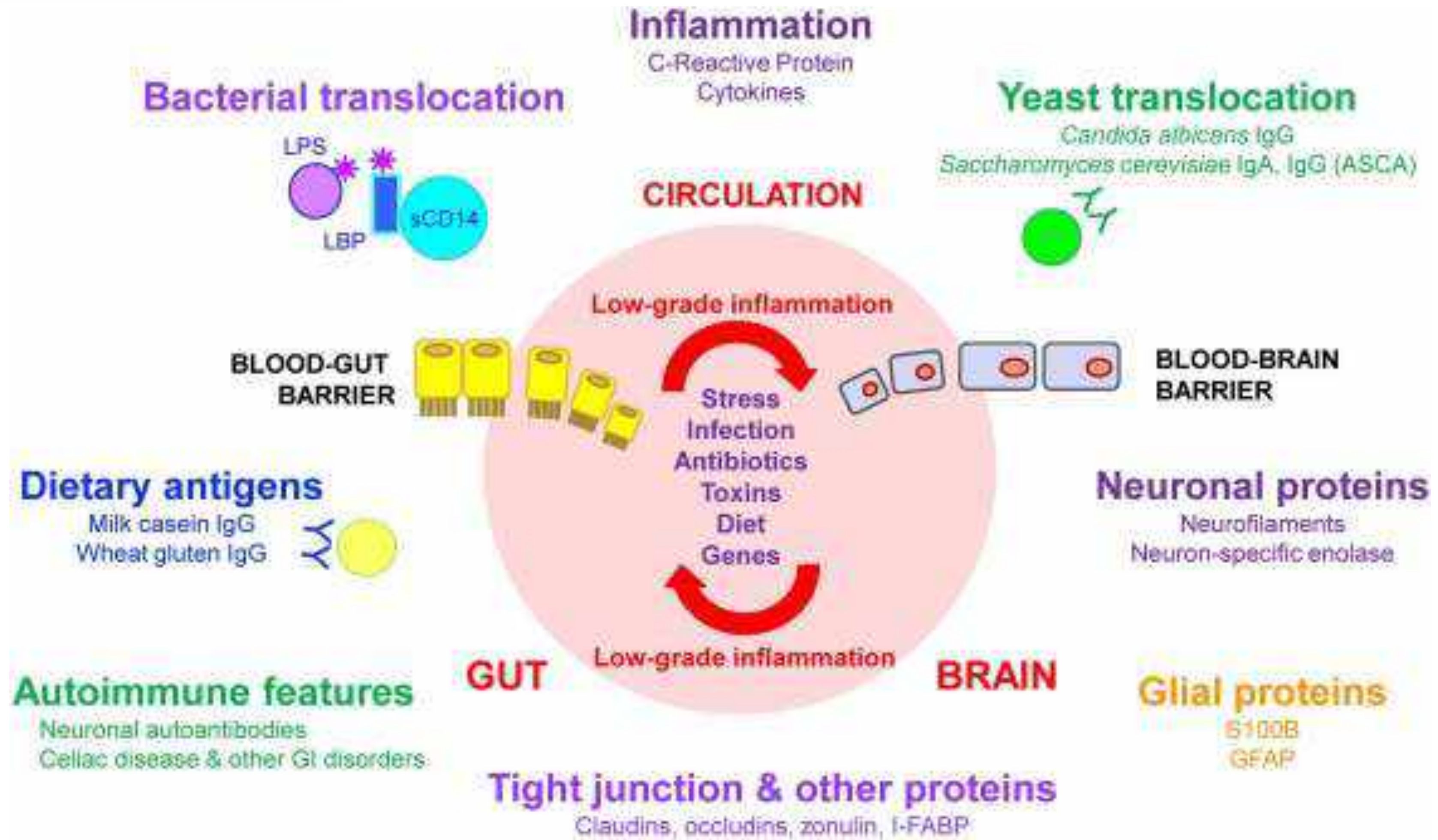
Other biological measures

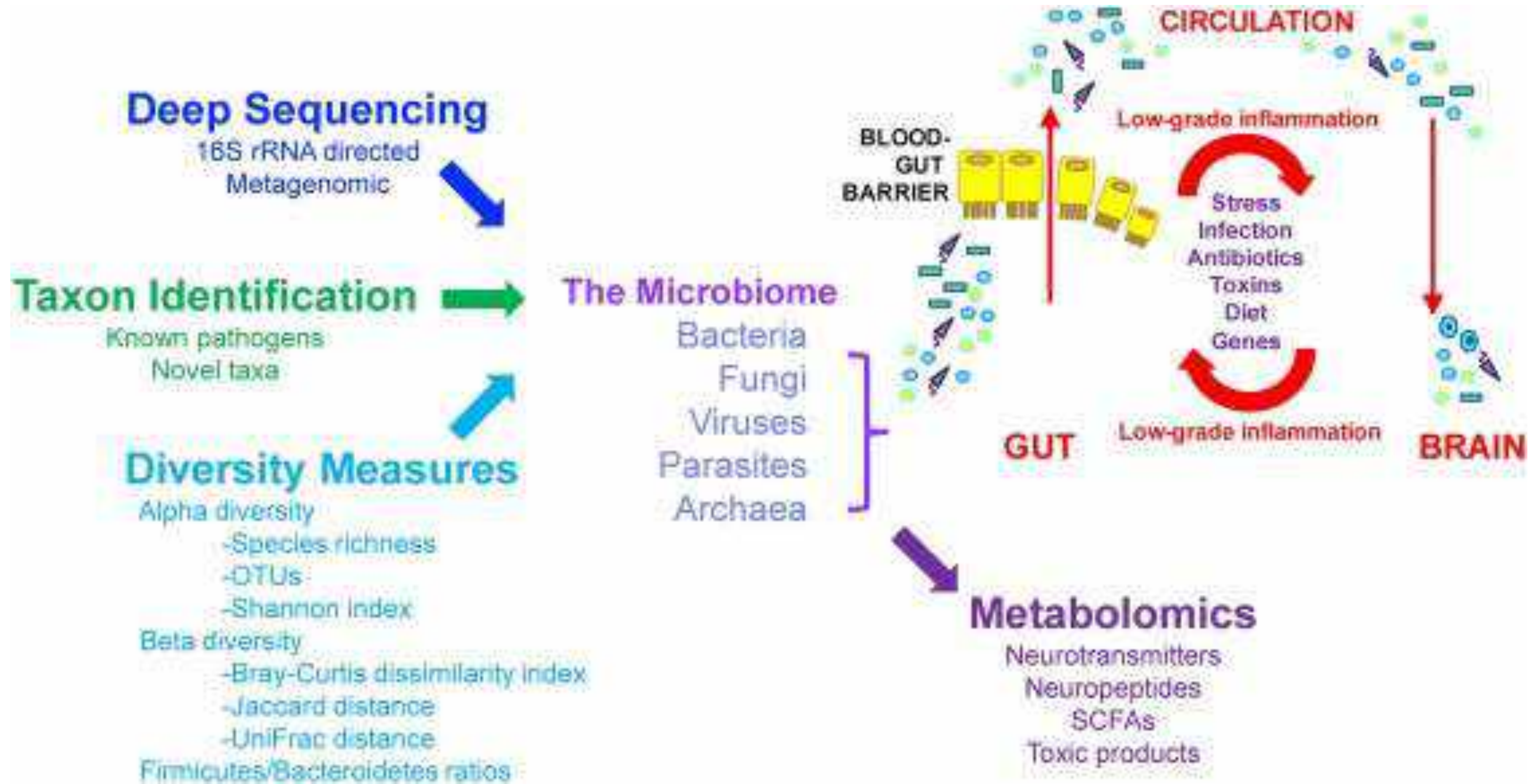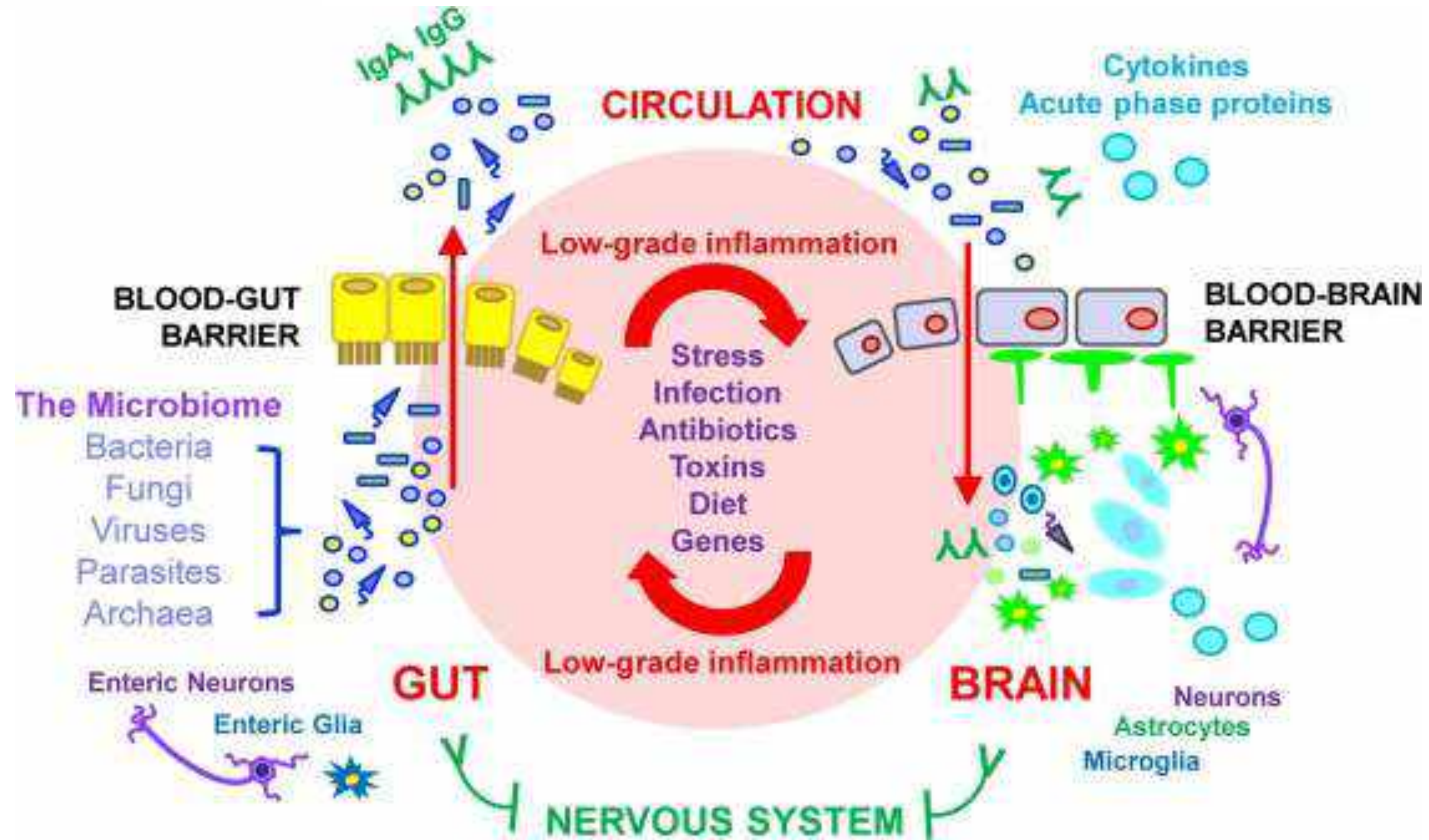| Probiotic	Duration	N	Subjects	Design	Clinical measures	Biological measures	Results	References
Lactobacillus casei Shirota (milk drink)	21 days	124	Healthy (average age 61.8 years)	Randomized double blind placebo controlled	Mood: profile of Mood States (POMS), at baseline, 10 days and 20 days Cognition: Episodic memory Semantic memory Verbal fluency	N/a	No general effect on mood of taking the probiotic Small improvement in mood when post-hoc analysis of the lowest tertile mood scores were considered Decreased performance on semantic memory	Benton et al., 2007
L. helveticus R0052 and B. Longum R0175	30 days	30	Healthy	Double blind placebo controlled	Hopkins Symptoms Checklist (HSCL-90) Hospital Anxiety and Depression Scale (HADS) Perceived Stress Scale (PSS) Coping Checklist (CCL)	24 h Urinary free cortisol (UFC)	Reduced global severity index, somatisation, depression and anger–hostility scores in the HSCL-90 Reduced global and anxiety scores in the HADS Improved problem solving in the CCL Decrease in UFC	Messaoudi et al., 2011
Lactobacillus casei Shirota	60 days	35	Chronic Fatigue Syndrome	Randomized double blind placebo controlled	Beck Anxiety and Depression Inventories	Fecal	Decrease in Anxiety symptoms Increase in Lactobacillus and Bifidobacteria in Fecal samples	Rao et al., 2009
Clostridium Butyricum	14 days (twice daily)	30 20 Healthy controls	Pre-op laryngectomy	Randomized, placebo controlled	Hamilton Anxiety Scale (HAMA)	Serum CRF Heart rate (HR)	Reduced anxiety levels from 19.8 to 10.2 in the HAMA Attenuated the increase in CRF and HR pre op	Yang et al., 2014
Bifidobacterium animalis, Streptococcus thermophiles, Lactobacillus bulgaricus, and Lactobacilluslactis (fermented milk)	28 days	12	Healthy Females	Randomized placebo controlled parallel-arm design		fMRI: emotional faces attention task	Reduced task related response of a distributed functional network containing affective, viscerosensory and somatosensory cortices independent of self-reported GI symptoms	Tillisch et al., 2013
Bifidobacterium bifidum W23, Bifidobacterium lactis W52, Lactobacillus acidophilus W37, Lactobacillus brevis W63, Lactobacillus casei W56, Lactobacillus salivarius W24, and Lactococcus lactis (W19 and W58)	28 days	40	Healthy	Triple-blind, placebo-controlled, randomized	Leiden index of depression sensitivity scale	N/a	Reduction in rumination and aggressive thoughts, subscales on the Leiden index of depression sensitivity scale	Steenbergen et al., 2015

Gut microbiome and serum metabolome analyses identify molecular biomarkers and altered glutamate metabolism in fibromyalgia

M. Clos-Garcia et al. / EBioMedicine 46 (2019) 499–511
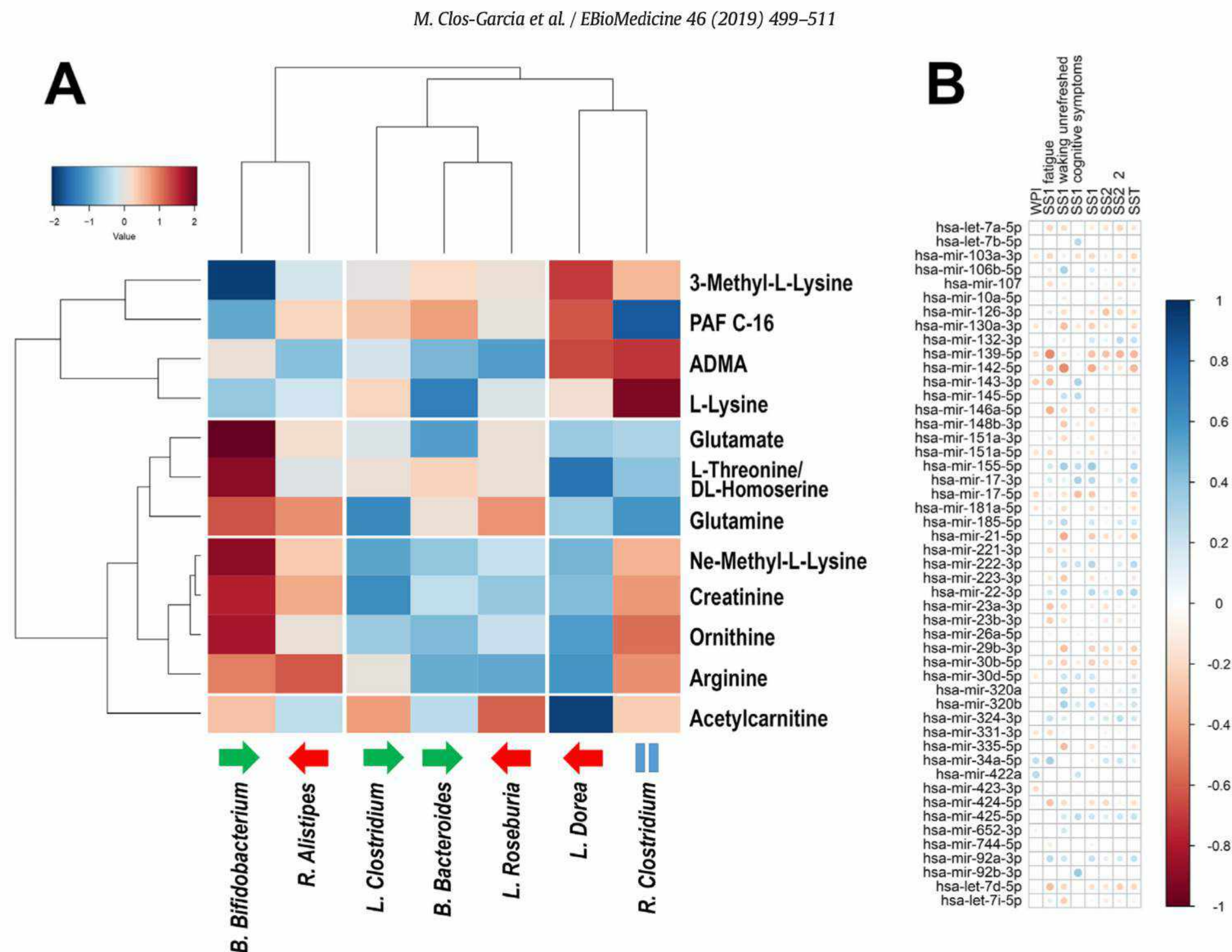
Example of metabolic analysis, i.e. small size molecules metabolised by gut microbiota, hence the importance of choosing the appropriate analysis method.

Effects of a Psychobiotic Supplement on Serum Brain-derived Neurotrophic Factor Levels in Depressive Patients: A *Post Hoc* Analysis of a Randomized Clinical Trial

Nazanin Heidarzadeh-Rad [1], Hülya Gökmen-Özel [1], Asma Kazemi [2], Negin Almasi [1], Kurosh Djafarian [3]

Methods: Our study was a double-blind, randomized controlled trial of patients with low-to-moderate depression receiving either a probiotic combination, prebiotic or placebo. From the 110 patients randomized in the trial, 78 were included in this *post hoc* analysis (probiotic, n = 28; prebiotic and placebo, n = 25). We compared serum BDNF levels from participants at baseline and endpoint, and assessed the Pearson correlation between depression severity and BDNF levels for each intervention.

Conclusion: Eight-week supplementation with B. longum and L. helveticus in depressive patients improved depression symptoms, possibly by increasing BDNF levels.

**Serum Brain-derived Neurotrophic Factor (BDNF) is a growth factor that has been seen to correlate with anti-depressant response in depressive patients.**

In this section I've summarised what I consider to be the key methodological considerations for successful gut-brain axis clinical trials.

More methodological considerations

Considerations for participant enrolment and data collection

Springer
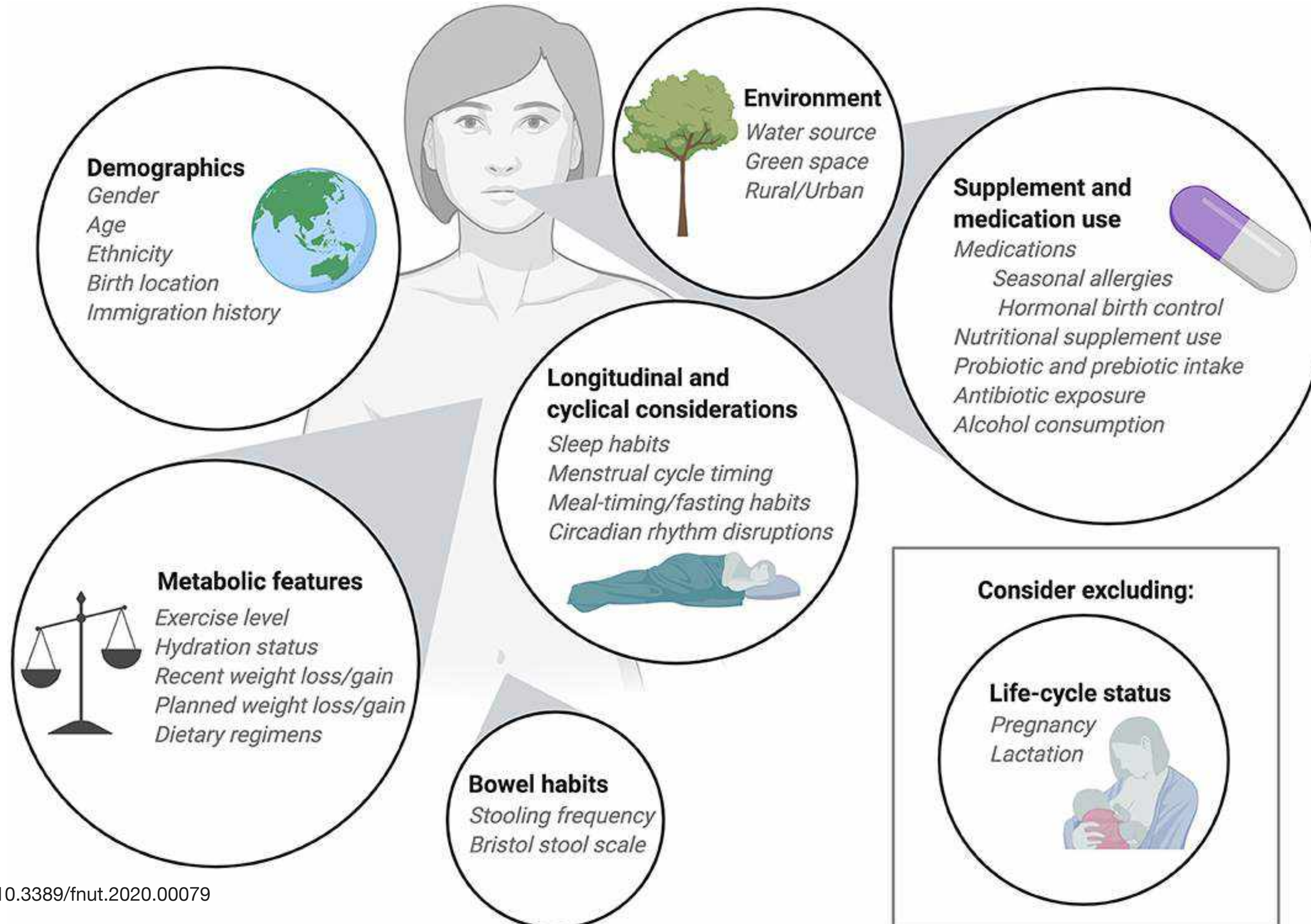
Sex Differences in the Gut-Brain Axis: Implications for Mental Health

Calliope Holingue [1] [2] [3], Alexa Curhan Budavari [4], Katrina M Rodriguez [4], Corina R Zisman [5], Grace Windheim [6], M Daniele Fallin [4]
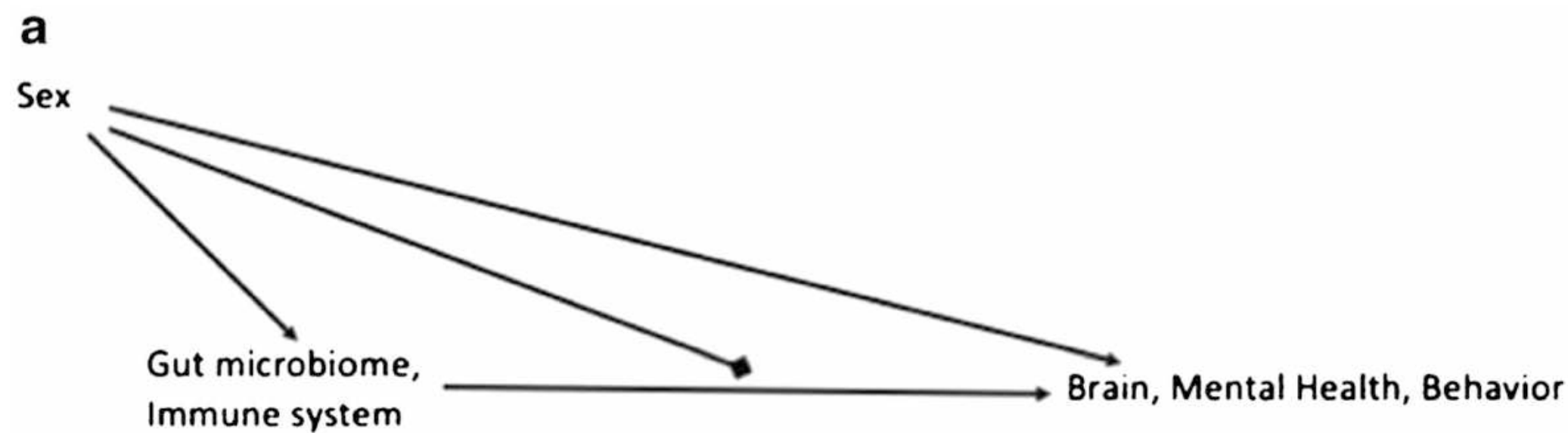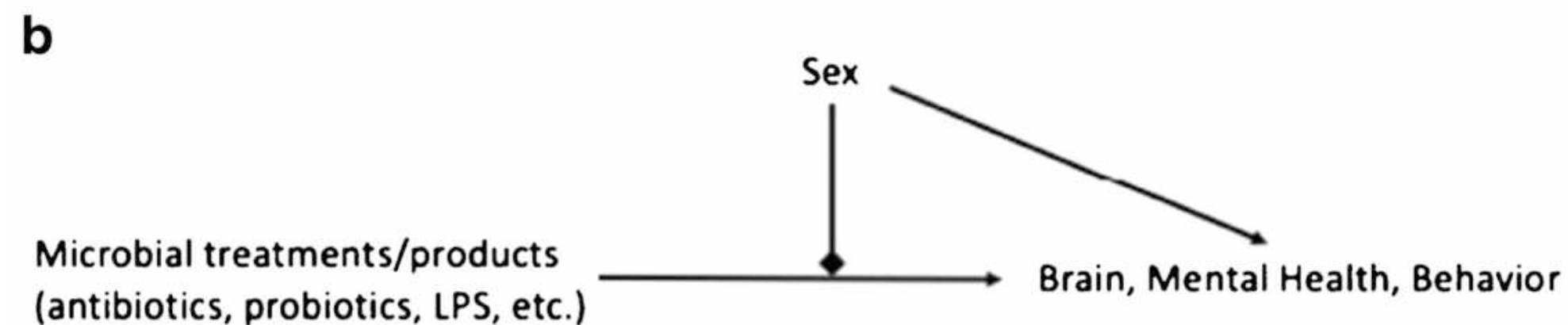
Gender split. There is emerging evidence that assessing the role of sex in the gut-brain axis may help elucidate the aetiology of and identify effective treatments for neurodevelopmental, psychiatric, and neurodegenerative disorders.

Stringent and clear inclusion and exclusion criteria

ORIGINAL ARTICLE

Bacillus coagulans MTCC 5856 for the management of major depression with irritable bowel syndrome: a randomised, double-blind, placebo controlled, multi-centre, pilot clinical study

Muhammed Majeed[1,2,3,4], Kalyanam Nagabhushanam[2], Sivakumar Arumugam[1], Shaheen Majeed[2,3] and Furqan Ali[1]*

food & nutrition research

Table 2. Demographics and baseline clinical characteristics

	Placebo (n = 20)	Bacillus coagulans MTCC 5856 (n = 20)
Sex, n (%)		
Female	17 (85)	17 (85)
Male	03 (15)	03 (15)
Age (years), mean (SD)		
	43.88 ± 9.85	40.36 ± 10.28
Height (cm), mean (SD)		
	157.39 ± 8.49	160.1 ± 7.87
Body mass index (kg/m²)		
	25.9 ± 4.49	25.4 ± 4.46
Smokers, n (%)		
Ex-smoker	18 (90)	19 (95)
Non-smoker	01 (5)	00
Smoker	01 (5)	01 (5)
Race, n (%)		
Central American	00	00
East Asian	00	00
South Asian	20 (100)	20 (100)
South American	00	00
South East Asian	00	00
Western European	00	00
White	00	00
Alcohol use		
Non-drinker	01 (5)	00
Past drinker	18 (90)	19 (95)
Occasional drinker	01 (5)	01 (5)
Current drinker	00	00

Inclusion criteria

1. Male and/or female subjects ranging in age between 20 and 65 years.
2. Fulfilling Rome III Diagnostic Criteria (30) for Functional IBS. Criterion fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis:
   a. Discomfort or recurrent abdominal pain at least 3 days/month in the last 3 months associated with two or more of the following: improvement with defecation, stool frequency change and change in appearance of stool
   b. Bloating or visible distension at least 3 days/month in the last 3 months
   c. Watery or loose stools without pain occurring in at least 75% of stools
3. Willingness to follow the protocol requirement as evidenced by written informed consent.
4. Diagnosed patients with mild to moderate IBS in severity with possible sleep, pain and dementia-associated co-morbidities.
5. Fulfilling Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (2000) Criteria for MDD.
6. Willingness to complete subject diaries and study questionnaires.
7. Agree not to use any medication (prescription and over the counter), including vitamins and minerals, during the course of this study.
8. Agree not to use any yogurt during the course of this study.
9. Subjects whose blood chemistries are within a normal range or not considered clinically significant if outside the normal range.
10. Subject's assurance that they have not taken antibiotics or other supplements whose primary site of action is in the gastrointestinal tract for a period up to 1 month prior to the start of the study.
11. Willing to come for regular follow-up visit.

Exclusion Criteria

1. Any clinically significant medical history, medical finding or an ongoing medical condition exists which in the opinion of the investigator could jeopardise the safety of the subject, impact validity of the study results or interfere with the completion of study according to the protocol.
2. Significant abnormal findings as determined by baseline history, physical examination, vital signs, haematology, serum chemistry and urinalysis.
3. History or presence of significant alcoholism or supplement/drug abuse in the past 1 year.
4. Any medical or surgical conditions which might significantly interfere with the gastrointestinal tract, liver, kidneys and/or blood-forming organs.
5. History of cardiovascular, renal, hepatic, asthma, glaucoma, pulmonary, neurologic, metabolic or psychiatric disease.
6. Participation in a clinical study during the preceding 90 days.
7. History of malignancy or other serious disease.
8. Any contraindication to blood sampling.
9. Smoking or consumption of tobacco products.
10. Blood or blood products donated in past 30 days prior to study supplement administration.
11. Pregnant female subjects and lactating women.
12. Prior surgical therapy for obesity.
13. Patients using yogurt in their daily meal.

Fundamental considerations for studying, analysing and interpreting gut microbiome data:
What kind of analysis?

Broad view					Narrow view
Metabolomics (Non-protein small molecules) All small molecules made by all organisms present Targeted: better for known metabolites (i.e. bile acids) Non-targeted: better for novel compounds, discovery Good for looking at functional changes No link to specific organisms	**Metaproteomics** (Protein) **Metatranscriptomics** (RNA) All protein or RNA made by all organisms present Good for looking at functional changes No link to specific organisms	**Shotgun sequencing** (Complete genomes, "Metagenomics") Every organism present will have most of the genomes sequenced: all bacteria fungi, viruses, etc. This includes the host/patient, discuss if using biopsy samples All organisms present No functional changes	**Amplicon sequencing** (Partial genomes) Most selected organisms present, depending on method used (no viruses) Most selected organisms present - uses 16S, 18S or ITS as "barcode"	**PCR panels** (qPCR, RT-PCR) Can include a single type or a select combination of organisms Generally up to around 24 per sample Limited in scope to known specific organisms in selected panel	**Culture** (traditional method for bacteria; also some archaea and viruses) A small number of known organisms that will grow on specific media under aerobic conditions Anaerobes can be isolated and grown, but many difficulties are present Limited in scope to known organisms under specific conditions
All organisms (including host)	RNA viruses and all organisms (including host)	All organisms (including host)	Bacteria and some archaea for 16S Eukaryotes only for 18S Fungi only for ITS	Viruses and other selected organisms (depends on panel used)	Bacteria, fungi, archaea and viruses (depends on media used)
High throughput 96 samples per run ~ 48 hours $$$	High throughput 96-384 samples per run ~ 48 hours $$$$	High throughput 384 samples per run 48 hours $$$	High throughput 384 samples per run 48 hours $$	Low throughput Max 30 pooled samples per run 1 to 5 hours $$	Low throughput 1 sample per media used 24 to 48 hours $

*"Convention dictates that a level of statistical significance of 5% and a statistical power of 80% are generally accepted values for the majority of studies. We would therefore recommend enrolling a total of **110 patients (55 per group)** to detect differences in alpha diversity of ≥2 units. It is worth noting that the logistics involved in recruiting 55 patients with a particular clinical phenotype may prove challenging, if not impossible, within the timeline available for some pilot studies. In addition properly accounting for additional factors such as medication, age, diet, or body mass index may further complicate this task. It is sensible, in these situations, to settle for a larger effect size; in the example provided, a total sample size of **50 patients** may be sufficient for an effect size of 0.80 (ie, a mean difference of 3 Faith PD units), at the risk of failing to detect real but smaller effects."*

Key points

Sample sizes in microbiota-gut-brain axis studies range from 50 to 100 participants. With these smaller sample sizes, the use of patient-reported outcome measures (PROMs) becomes indispensable. There are a range of such instruments reported in literature, helping researchers to assess stress, anxiety, depression and various different domains of cognitive function. Electronic tools are preferred but many instruments provide paper versions.

Onset of action is often difficult to ascertain, given that most studies only report baseline and post-intervention measures. Most interventions range from 4 to 12 weeks. Ideally clinical trials should consider assessing intervention effects at interim points in time, e.g. every 4 weeks in a 12 week trial. This would provide clinicians with an opportunity to draw richer insights that can help them assess the usability of health products.

Questions & Answers

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for inviting me to share my knowledge and expertise with you.

Adri Bester, Academic Lead at the London Agri-Food Innovation Clinic, Enterprise Lead for the School of Applied Sciences, London South Bank University.

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