Gut microbiota and probiotic therapy in ADHD: A review of current knowledge

Anna Kalenik [*], Karolina Kardaś, Anna Rahnama, Katarzyna Sirojć, Tomasz Wolańczyk

Department of Child Psychiatry, Medical University of Warsaw, Poland

ABSTRACT

Attention deficit hyperactivity disorder (ADHD) is a common neurodevelopmental disorder characterized by inattention, impulsivity and hyperactivity. The etiology of the disorder is multi-factorial, with a main focus on genetic factors. However, emerging research shows the involvement of changes and imbalances in the intestinal microbiota. Evidence for the influence of gut microbiota on brain development and neurogenesis is clear.

We present a review of emerging research on the microbiota in the ADHD population. The aim of this study was to summarize the current state of knowledge on ADHD, to identify gaps in knowledge, as well as to indicate the directions of new research. Thanks to the researchers that would be possible to better understand the complexity of ADHD etiology, especially the role of the intestinal microbiota in the pathogenesis of the disorder. Pubmed, Scopus and Google Scholar databases were used while writing the review.

Numerous studies show that probiotic supplementation can have a positive effect on the course of neurodevelopmental disorders, including ADHD. Unfortunately, clinical studies that were identified are mostly inconclusive, and more high-quality research is needed to produce robust evidence for therapy based on interventions targeting microbiota.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by a continuous disorder of the cognitive functioning pattern, clinically manifesting itself primarily with symptoms that could be divided into three groups: excessive mobility, inattention, and excessive impulsiveness. The symptoms are developmentally inappropriate and highly impairing. ADHD is one of the most common neurodevelopmental disorders diagnosed in children and adolescents, however, it should not be forgotten that this disorder also affects a large group of adult people. Studies have shown that pooled estimates suggest that 7.2% of children are affected by ADHD (Thomas et al., 2015).

The etiology of attention deficit hyperactivity disorder is complex – a number of factors have been demonstrated that may affect the occurrence of the disorder, such as genetic and environmental factors and those associated with perinatal damage; however, identifying true causality of environmental risk factors for ADHD is highly disputable. There is a well-known genetic predisposition shown in numerous family and twin studies (Grimm et al., 2020). Studies conducted on pairs of twins show a very high level of inheritance, estimated at 71–90% (Faraone et al., 2005).

The most important role is attributed to genetic factors, mainly associated with genes for the dopamine DRD4 and DRD5 receptors as well as genes for the dopamine and serotonin transmitter (Bonvicini et al., 2020), and the dopaminergic theory of ADHD will be disputed below. Genome-wide association studies (GWAS) have discovered a relationship between microdeletion and microduplication of DNA fragments that are typically not seen in karyotype tests (Byrne et al., 2020). However, no specific genes have been identified that could be directly responsible for the development of ADHD (Grimm et al., 2018). On the other hand, a recent meta-analysis revealed 12 independent genome-wide significant loci and found that *FOXP2* on chromosome 7 correlates with the diagnosis (Demontis et al., 2019).

Another new direction of focus of studies on the risk factors for attention deficit hyperactivity disorder is changes and imbalances in intestinal microbiota and its potential impact on the occurrence and clinical course of neurodevelopmental disorders (Sandgren and Brummer, 2018).

The main problems discussed in our review are the subject of the

* Corresponding author.
  E-mail address: anna.kalenik@uckwum.pl (A. Kalenik).

broadly understood intestinal microbiota in the context of ADHD, particularly its impact on the frequency and severity of the symptoms of this neurodevelopment disorder, the theoretical basis of the observed correlations and the hypothetical therapeutic possibility of probiotics.

The method used was review of the available international literature from 1999 to 2020 with particular emphasis on the news of the last 10 years.

We have reviewed the literature on the influence of the gut microbiota on the function of the dopaminergic and noradrenergic systems, we thoroughly described the observed changes in the intestinal flora in ADHD patients, as well as we have reviewed and summarized the latest scientific reports on modifying the composition of the microbiota and the potential therapeutic effect of the above-mentioned.

Pubmed, Scopus and Google Scholar databases were used while writing the review. The work presents the state of knowledge on October 20, 2020. We have used keywords: ADHD, attention deficit hyperactivity disorder, gut microbiota, probiotic, gut-brain axis and their appropriate combinations to find the relevant papers. We have included 79 literature positions. In order to present the results clearly, we have prepared adequate tables and a comprehensive summary with the conclusions of the review.

2. The dopaminergic and noradrenergic system and microbiota

The association of intestinal microbiota with the aetiopathogenesis of various disease entities has been the subject of intense research interest for several decades. In addition to the vagus nerve and the immune system, the intestinal microbiota, as a component of the "gut-brain axis", plays a role in the bidirectional communication between the intestines and the central nervous system. As this complex bacterial community contains more than 100 trillion microorganisms, the microbiota is known not only for the direct possibility of modulating and producing neuroactive compounds and neurotransmitters (Dinan and Cryan, 2015; Martin et al., 2018; Strandwitz, 2018) but also via the hypothalamic-pituitary-adrenal (HPA) axis (Bonaz et al., 2018), secretion of short-chain fatty acids and portability of the blood-brain barrier (Spadoni et al., 2015). In contrast, brain activity affects the population and composition of gut microbiota (Petra et al., 2015). Those studied pathways confirm mutual interaction between microbiota and central nervous system (Fig. 1).

There is clear evidence of the influence of gut microbiota on brain development and neurogenesis (Heijtz et al., 2011). Studies have

already shown influences on neuropsychiatric disorders such as depression (Kelly et al., 2016), schizophrenia (Dinan and Cryan, 2015), autism spectrum disorder (Hsiao et al., 2013), irritable bowel syndrome (Didari et al., 2015), Parkinson's disease (Sampson et al., 2016), and Alzheimer's disease (Minter et al., 2016) both in studies on rodents and humans. Bacteria are known for their capability to produce neurotransmitters: dopamine, norepinephrine, serotonin, and GABA, and their precurosors (i.e. tyrosine, tryptophan, phenylalanine) as well as their impact on their metabolism (Lyte, 2016; Strandwitz, 2018).

Studies in ADHD have shown structural, functional, and developmental alterations mainly in the frontostriatal circuitry, cerebellum, temporoparietal lobes, basal ganglia, and corpus callosum (Cortese, 2012). As mentioned before, theories about the neurobiology of ADHD are focusing on dysfunctions mainly around noradrenergic and dopaminergic but also serotoninergic and cholinergic pathways (Tripp and Wickens, 2009).

The dopaminergic system is the basis for ADHD symptoms, including cognitive, emotional, and behavioural aspects (Cortese, 2012). Dopamine, synthesized in the brain and kidneys, is not a precursor only for catecholamines but is also a reward-motivated behavior and executive function neurotransmitter. Both low and high levels of norepinephrine diminish information processing capacity as well as selective attention capacity (Berridge and Stalnaker, 2002). Located in the dorsolateral prefrontal cortex, alpha-2 adrenergic receptors inhibit the control of locomotor activity, decrease the availability of dopaminergic receptors (D2, D3) and increase dopamine transporter binding (Ma et al., 2005; Prince, 2008). Serotonergic and cholinergic systems have also been shown to impair neurochemical changes in ADHD. Trough among others dopaminergic neurotransmission modification serotonin alters learning and memory function and may have an impact on emotions, stress response, appetite, and addiction (Seyedabadi et al., 2014).

The dopamine hypothesis highlights that the brain may be affected by enteric neurotransmission of this neurotransmitter being released after nutrients stimulate this tract (de Araujo et al., 2012).

There is evidence that the microbiome can influence dopamine levels in the frontal cortex and striatum in rodents (Bercik et al., 2011). The study of the gut microbiome axis revealed that the level of cyclohexadienyl dehydratase involved in phenylalanine production (dopamine precursor) remains correlated with the abundance of the genus Bifidobacterium in the microbiome of patients with ADHD (Aarts et al., 2017). Numerous bacteria have also been known for their ability to produce dopamine and noradrenaline in vitro directly i.e. *Bacillus*
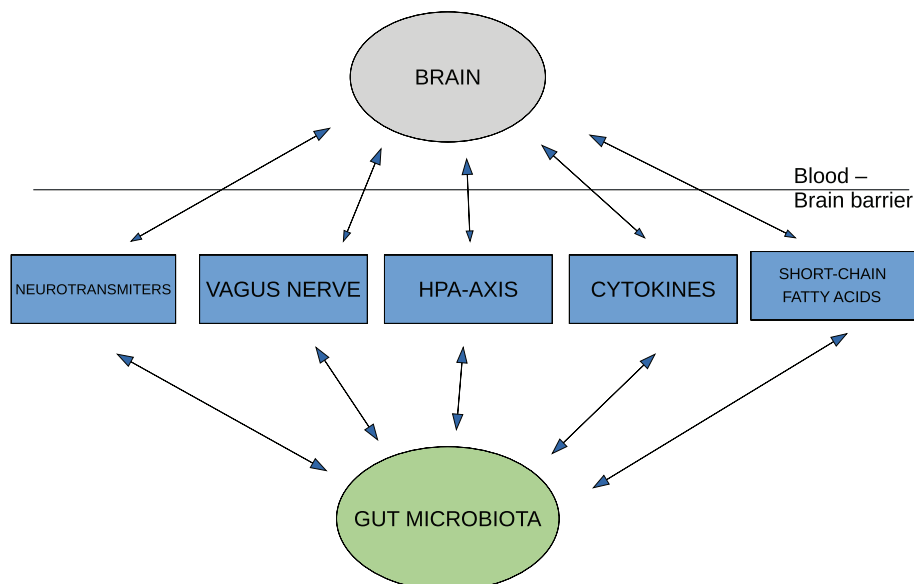

Fig. 1. The gut-brain axis

subtilis, Bacillus mycoides, E. coli, Proteus vulgaris, Serratia marcescens (Tsavkelova et al., 2000). Findings also suggest that Clostridia species influences lumen levels of norepinephrine (Asano et al., 2012) while it's not clear yet, whether production is direct or bacteria are responsible of modulating host production. The proposed model of autonomic dysregulation in ADHD emphasizes dysregulation of vagus nerve dysregulation, and both afferent and efferent signalling in addition to altered immunoregulation might be an answer as to how the cognitive, emotional, and behavioural symptoms of ADHD emerge.

Since the microbiome can be manipulated both by probiotic modulations and fecal transplant, identifying specific bacteria for the therapeutic approach is opening up a wide range of possibilities in the development of new treatments (Mathee et al., 2020). From this point it is crucial to investigate microbiota composition in ADHD and its relationship with patients symptoms.

3. Gut microbiota in ADHD

3.1. Research review

In recent years, several clinical studies have focused on finding relationships between the composition and diversity of the intestinal microflora and the presence of symptoms of ADHD and their severity. Only 10 publications on this subject have been published so far. They are presented chronologically as to the date of publication in Table 1.

The first publication that contained a determination of the differences in the microbiome among ADHD patients appeared relatively recently, in 2015. Pärtty et al. published a study that examined the composition of the intestinal flora in children born in Finland. It was shown that at 3 months of age, patients who were later diagnosed with ADHD and ASD had less *Bifidobacterium longum* than healthy children. In the sixth month, this difference was even more pronounced. At 18 months, they also had fewer *Bacteroides* and *Lactobacillus-Enterococcus* bacteria and at 24 months, fewer *Clostridium histolyticum* cells compared to children without subsequent diagnosis. Interestingly, repeated examination of the composition of the intestinal microbiome in 13-year-old ADHD cases and in the controls did not show statistically significant differences between the two groups. On the basis of this publication, it could be hypothesized that the greater amount of *Bifidobacterium longum* in younger children may indicate a lower risk of ADHD in the future. However, results of studies on this topic are contradictory (Aarts et al., 2017; Stevens et al., 2019). The limitation of this study is undoubtedly a very small group of children diagnosed with ADHD - only 5 participants (two of them with coexisting ASD). It is also worth paying attention to the methods of microbiome analysis used - qPCR and FISH. Therefore, the researchers did not profile the entire microbiome, but only focused on selected groups of bacteria. All subsequent studies used newer and more accurate analysis techniques.

Aarts et al. (2017) focused on examining the complete intestinal microbiological using a method that enabled them to obtain a full microbiome profile (16S rRNA gene sequencing). An increase in bacteria from the Actinobacteria taxon was observed (22.14% ADHD and 14.08% control). The Actinobacteria cluster includes the genus *Bifidobacterium*, which has been observed to increase significantly in patients with ADHD, as opposed to the results reported by Pärtty et al. (2015). In both groups, the Firmicutes type was the most numerous, while the percentage of Firmicutes was smaller in the ADHD cases than in the control group (70.3% in ADHD and 79.8% in the control), but no specific type responsible for this result was identified. However, the phylum Bacteroidetes did not differ significantly. The authors also noted the correlation between increased *Bifidobacterium* with diminished ventral striatal fMRI responses during reward anticipation. To check whether the diagnosis of ADHD has an effect on alpha diversity (i.e., how many different species of intestinal microflora can be found) the Shannon index was used. Its greater value in patients with ADHD testified to the greater species diversity of bacteria in this group. This is the only one

research that used additional imaging studies, but only 6 patients with ADHD had both a microbiome and fMRI analysis done simultaneously.

Prehn-Kristensen et al. (2018) showed that in ADHD cases, the level of alpha diversity was significantly lower than in the control group. In addition, they observed a significant correlation between reduced diversity and the severity of hyperactivity. Researchers did not observe significant differences at the phylum level. However, patients with ADHD were found to have a larger abundance of the genera *Bacteroides* and *Neisseria* in relation to controls and *Bacteroides* spec. correlated with levels of hyperactivity and impulsivity. In this study, no significant effect of psychostimulant treatment on the diversity of intestinal flora was demonstrated. It is also the only study to include parents of children with ADHD. Mothers of ADHD patients showed reduced alpha diversity compared to mothers of healthy controls, and differed in beta diversity compared to both groups of children. There were no significant differences between fathers and children in alpha diversity. According to the authors, this may indicate that the composition of the flora is not inherited from mothers. However, there is insufficient evidence to support this hypothesis. Due to these differences, it may be useful to include parents in future research.

In the next study, the results were different again. Chinese authors (Jiang et al., 2018) did not show differences in alpha diversity. In order to exclude the influence of drug on the results, only treatment-naïve patients were qualified. However, researchers noticed a significantly reduced amount of *Faecalibacterium* (in the phylum Firmicutes) in patients with ADHD, and there was a negative correlation between that amount and the severity of symptoms reported by parents. They proposed *Faecalibacterium* as a new probable marker of ADHD.

A completely different method was used by the authors of another study (Cheng et al., 2020). They used bioinformatics techniques, namely Gene Set Enrichment Analysis (GSEA). It is a computational method by which they compared the relationships of the gut microbiota with five psychiatric diagnoses, including ADHD. They analyzed information from the genome-wide association study (GWAS) and gut microbiota data. They observed that ADHD is associated with an increase in the genus *Desulfovibrio* and order Clostridiales. However, these were not specific for this neurodevelopmental disorder. Changes in *Desulfovibrio* genus were also associated with four other diagnoses, and Clostridiales with another. Considering the current development of technology and bioinformatics, we can expect more detailed and extensive research of this type in the future.

In the same year, in Taiwan, Wang et al. (2020) found significantly higher Shannon and Chao indices in the ADHD group, but other alpha-diversity indices (Simpson index, ACE index) were lower or insignificant, respectively, compared to the control group. The variability of several bacterial species was observed; the amount of *Bacteroides corpocola* was smaller in the ADHD group, while *Bacteroides uniformis*, *Bacteroides ovatus*, and *Sutterella stercoricanis* were more numerous. A positive correlation with the severity of symptoms was shown for the last two species. In both groups, intestinal microbiome profiles were similar, with Bacteroidetes dominating at a similar level. The authors also noted an increase in *Fusobacterium* (0.28% in ADHD and 0.02% in control). They identified three species (*Bacteroides uniformis*, *Bacteroides ovatus* and *Sutterella stercoricanis*) as potential biomarkers of ADHD. This is partly consistent with the indicator proposed by Prehn-Kristensen et al. (2018). The strength of this study is also the use of a detailed food frequency questionnaire (FFQ). This allowed for the association of the species of intestinal bacteria with the food consumed in cases of ADHD and at the same time confirmed the already known effect of food on the intestinal microflora. *S. stercoricanis* was dependent on the consumption of dairy, nuts, seeds, legumes, ferritin, and magnesium. In turn, *B. uniformis* was associated with the frequency of consumed carbohydrates and fat.

Stevens et al. (2019) from New Zealand compared the effect of micronutrient supplementation on the gut microbiome between two groups of patients with ADHD. They conducted a 10-week randomized

Table 1
Characteristics of included studies

Study	Population characteristics Cases	Controls	Diagnostic instrument	Diet	Medications and supplements	Probiotics	Antibiotics	Comorbidities	Method	Sample	Sample collection and storage	DNA extraction	Sequencing technique	Data processing/Database	Results cases versus controls Differences	alpha-diversity	beta-diversity	Authors' conclusions
Pärtty et al. (2015), Finland	ADHD/AS, n=6*; Males: 100% *Composition of the ADHD/AS group: ADHD, n=3 AS, n=1 ADHD+AS, n=2	n=69 Males: 49%	ICD-10		methylphenidate, n=4; ω–3 fatty acid supplementation, n=2	Lactobacillus rhamnosus G G for 4 weeks before delivery and for 6 months after	Antibiotic treatment during the first 6 mo of life: ADHD/AS: 1 Healthy: 19		FISH and qPCR	Fecal samples	at 3 wk, 3, 6, 12, 18, 24 mo, 13 y; at hospital or at home, 4°C, delivered to the hospital <24 h, then −80°C	KingFisher DNA extraction, InviMag Stool DNA kit, then -20°C	ABI PRISM 7300-PCR detection system		3 mo: ↓Bifidobacterium; 6 mo: ↓Bifidobacterium; 18 mo: ↓Bacteroides and Lactobacillus-Enterococcus; 24 mo: ↓Clostridium histolyticum; 13 y: NS; At the age of 13 y, ADHD or AS was diagnosed in 6/35 (17.1%) children in the placebo and none in the probiotic group			Early supplementation with Lactobacillus rhamnosus GG may reduce the risk of ADHD
Aarts et al. (2017), The Netherlands	ADHD, n=19 19,5 yo BMI: 23,8 Males: 68,4%	n=77 27,1 yo BMI: 23,0 Males: 53,2%	K-SADS-PL, DSM-IV						16S rRNA	Fecal samples	in a 50ml Falcon tube, 4°C, then -80°C within 24 hours	DNeasy® Blood and Tissue Kit (Qiagen)	V3–V4 regions, 454 Life Sciences GS-FLX platform	QIIME version 1.2, The Ribosomal Database Project classifier version 2.3, Kyoto Encyclopedia of Genes and Genomes, PICRUSt	↓Firmicutes, ↑Bifidobacterium; The ADHD microbiome contains increased levels of predicted CDT (cyclohexadienyl dehydratase); The abundance of predicted CDT correlated negatively with reward anticipation responses in bilateral ventral striatum; Bifidobacterium was responsible for increases in the predicted function of CDT	Shannon index, Chao index: NS		An increase in Bifidobacterium was significantly associated with an increase in the predicted function of CDT (cyclohexadienyl dehydratase) and decreased ventral striatal fMRI responses during reward anticipation.
Prehn-Kristensen et al. (2018), Germany	ADHD, n=14 11,9 yo BMI: 19,0 Males: 100%	n=17 13,1 yo BMI: 18,0 Males: 100%	DSM-IV-TR, K-SADS-PL, CBCL, FBB-HKS	no differences in eating habits one month prior to sampling	Medikinet®, n=9 Equasym®, n=1; 10 patients taking medication > 1 year; 9 patients discontinued medications > 48h			ODD, n=6; other comorbidities were excluded	16S rDNA	Fecal samples	in Sarstedt fecal collection tubes, at 4°C until preparation	FastDNATM SPIN KIT FOR SOIL, after incubation in 200 ml Tris Lysisbuffer and 25ml proteinase K for 2 hours at 56°C, then -80°C	primers 27F-338R, V1-V2 regions, SequalPrep Normalization Plate Kit, Illumina MiSeq	Mothur	↑Bacteroidaceae, Neisseriaceae; ↑Neisseria; ↓Prevotellaceae, Catabacteriaceae, Porphyromonadaceae; ↓ Prevotella, Parabacteroides OTU_7 (Bacteroides spec.) correlated with levels of hyperactivity and impulsivity; negative correlation between level of hyperactivity and alpha diversity	Shannon index: ↑, Chao1: NS; Shannon index between treated MPH and without MPH: NS	significant differences between	Neisseria and Bacteroides as possible biomarkers of ADHD
Jiang et al. (2018), China	ADHD, n=51 8.47 yo BMI:16.4 Males: 74,5%	n=32 8.5 yo BMI:16.09 Males: 68,8%	DSM-IV, K-SADS-PL, CPRS	specific diet excluded, assessment of early feeding	treatment-naive		Antibiotics stopped >2 months	gastrointestinal symptoms, depressive or anxiety symptoms, obesity, common childhood atopic diseases were excluded	16S rRNA	Fecal samples	in sterile plastic cup at home, −20°C, shipped in cold pack within 30 min, then −80°C	QIAamp DNA Stool Mini Kit	primers 338F and 806R, V3–V4 regions, Illumina MiSeq	FLASH/UCHIME/QIIME	↓Faecalibacterium; negative correlation between Faecalibacterium and ADHD severity	NS		Faecalibacterium as possible biomarker of ADHD
Cheng et al. (2019), China	ADHD, n=19099	n=34 194							Bioinformatics techniques: Gene Set Enrichment Analysis (GSEA)					Genome-wide association studies (GWAS) from the Psychiatric GWAS Consortium	↑Desulfovibrio, Clostridiales			
Wang et al. (2019), Taiwan	ADHD, n=30 8,4 yo Weight (kg): 30.7 Males: 76,7%	n=30 9,3 yo Weight (kg): 35.6 Males: 60%	DSM-IV-TR, K-SADS-E, WISC-IV, SNAP-IV, ADHD-RS	vegetarians were excluded; food frequency questionnaire (FFQ) was used		taking probiotics excluded	taking antibiotics excluded	neuropsychiatric diseases and major physical illnesses were excluded	16S rRNA	Fecal samples	in a 50-ml Falcon tube, 4 °C immediately after collection and then at −80 °C within 24 h	QIAamp DNA Mini Kit	V3–V4 regions, Illumina MiSeq	Mothur v1.39.5/UCHIME/QIIME	↑Fusobacteria, Fusobacterium, Bacteroides uniformis, Bacteroides ovatus and Sutterella stercoricanis; ↓Lactobacillus and Bacteroides coprocola; B. ovatus and S. stercoricanis were positively correlated to ADHD symptoms; S. stercoricanis and B. uniformis were correlated with nutrients	Shannon index: ↑; Chao index: ↑; Simpson index: ↓; ACE index: NS	NS	The gut microbiome is associated with dietary patterns and susceptibility to ADHD; Bacteroides uniformis, Bacteroides ovatus and Sutterella stercoricanis as potential biomarkers of ADHD;
Stevens et al. (2019), New Zealand	the micronutrient treatment group ADHD, n=10 7-12 yo (mean 10,29) BMI: 19.39 Male: 100% ADHD symptoms: 41.6	the placebo group ADHD, n=7 7-12 yo (mean 9,3) BMI: 16.6 Male: 100% ADHD symptoms: 44.6	DSM-IV, CGAS, ADHD-RS-IV	a brief diet intake questionnaire	10-week dietary micronutrient supplementation with a blend of vitamins, minerals, amino acids and antioxidants (40 ingredients)		taking antibiotics during research excluded		16S rRNA	Fecal samples	the OmnigenEgut faecal collection system; at -4 °C for a maximum of 14 days, then at −80 °C	NucleoSpin DNA Stool isolation kit, Extracted DNA was stored at −20 °C until PCR amplification	V3–V4 regions, Illumina MiSeq	QIIME2, version 2017.12/CASAVA 1.8/DADA2/MAFFT/ANCOM/Tax4Fun/PICRUSt/the GreenGenes database/SILVA database	↓Actinobacteria (especially Bifidobacterium - B. longum and B. adolescentis); ↑Collinsella; Micronutrient supplementation was associated with a reduction in ADHD symptoms	↑observed OTUs in the treatment group; Shannon index, Faith's phylogenetic diversity: NS	NS	Bifidobacterium as being of potential relevance to ADHD
Wan et al. (2020), China	ADHD, n=17 6-12 yo (median 8 yo) BMI: 16,1 Male: 82,3%	n=17 6-12 yo (median 8 yo) BMI: 15,9 Male: 76,5%	K-SADS, Present and Lifetime Version scales, DSM-V, CPRS	special diet (e.g., vegetarian) were excluded; regular dietary patterns for a week; a food diary was recorded		no use of probiotics within 1 month		respiratory or digestive tract infection within 1 month, digestive diseases, allergic diseases, depressive or anxiety symptoms, BMI>20 were excluded	shotgun metagenomics sequencing	Fecal samples	at 8:00 am in the Pediatric Outpatient Department and stored in a sterile plastic cup at −80°C prior to testing	HiPure Stool DNA kits, Qubit 4.0, KAPA Hyper Prep Kit	Illumina NovaSeq platform	Integrated Gene Catalog databases, Kyoto Encyclopedia of Genes and Genomes	↑ Odoribacteraceae, Enterococcaceae; ↑Bacteroides caccae, Odoribacter splanchnicus, Paraprevotella xylaniphila, Veillonella parvula; ↓Ruminococcaceae, ↓Faecalibacterium and Veillonella, ↓Faecalibacterium prausnitzii, Lachnospiraceae bacterium, Ruminococcus gnavus; significant differences in the metabolic pathways of neurotransmitters	Shannon index, Chao1 index, Simpson index: NS		Differences in the gut microbiota can cause changes in the brain-gut axis and neurotransmitter levels, which may be associated with ADHD symptoms.
Szopinska-Tokov et al. (2020), The Netherlands	ADHD, n=41; subthreshold ADHD, n=15; 13-29 yo (mean 20.2 yo) BMI, median: 23	n=47 13-28 yo (mean 20,5 yo); BMI, median: 22 Male: 49%	K-SADS, DSM-IV, Conner s rating scale, CAARS, CTRS		Use of ADHD medication, n=19 (ADHD), n=3 (Subthreshold ADHD)				16S rRNA	Fecal samples	in a 50ml Falcon tube, 4°C, then -80°C within 24 hours	repeated bead-beating step, Maxwell® 16 Instrument	primers 27F-DegS and 338RI-II, V1-V2 regions, CleanPCR kit, Qubit® 2.0 fluorometer, Illumina HiSeq PE300	NG-Tax 16S rRNA pipeline, SILVA database, QIIME version 1.9.1	no significant differences at the phylum level; ↑nine genera (ex. Clostridiales_g__, Ruminococcaceae_UCG_004, Ruminococcaceae_NK4A214_group, Ruminococcus_2); ↓Haemophilus; Ruminococcaceae_UCG_004 and Ruminococcus_2 were associated with inattention; ↑Dialister and ↓Phascolarctobacterium in medicated ADHD	unique OTUs in each sample, Shannon index, Faith's phylogenetic diversity: NS	higher taxonomic similarity in ADHD	The Ruminococcaceae family may be associated with neurobiological processes and ADHD
	Male: 63% (ADHD), 40% (Subthreshold ADHD)																	

NS - non-significant, OUT - operational taxonomic unit, ↑ - increased, ↓ - decreased.

double-blind study. Patients were receiving supplementation with a mix of vitamins, minerals, amino acids and antioxidants - 40 ingredients in total. After micronutrient treatment, a reduction in ADHD symptoms was observed as well as improved attention. Functioning and regulation of emotions improved, aggression decreased. The analysis showed a reduction in Actinobacteria in the group with micronutrient supplementation. Two species in particular were responsible for this effect - *Bifidobacterium longum* and *Bifidobacterium adolescentis*. The association of *Bifidobacterium* with ADHD was also present in Aarts et al. (2017). Interestingly, Stevens et al. (2019) observed a negative correlation between the abundance of *Bifidobacterium* and the severity of ADHD symptoms, but only before supplementation. After 10 weeks, the result turned out to be contradictory as the correlation became positive. Nevertheless, the authors find *Bifidobacterium* as a possible factor in ADHD. However, this study did not show a relationship between supplement use and alteration of metagenomic pathways.

At the beginning of this year, more studies were published focusing on understanding the intestinal microflora of patients with ADHD symptoms.

Another Chinese group (Wan et al., 2020) found no significant difference in the microbial alpha diversity between ADHD cases compared to controls. However, differences were identified at the level of types and individual bacterial species. In the ADHD cases, the types of *Faecalibacterium* and *Veillonellaceae* (both belong to the phylum Firmicutes) were significantly reduced, while *Odoribacter* (Bacteroidetes) and *Enterococcus* (Firmicutes) were significantly increased. Regarding the specific species, in the ADHD group, *Faecalibacterium prausnitzii*, *Lachnospiraceae bacterium* and *Ruminococcus gnavus* (all belong to the phylum Firmicutes) were significantly decreased, whereas *Bacteroides caccae*, *Odoribacter splanchnicus*, *Paraprevotella xylaniphila* (all belong to the phylum Bacteroidetes) and *Veillonella parvula* (Firmicutes) were found in greater numbers. It is worth mentioning that this study examined the severity of constipation symptoms, which were more common in patients with ADHD (70.5% to 11.7%). Like Aarts et al. (2017), the authors also analyzed the metabolic pathways of neurotransmitters. They showed significant differences in the pathways of dopamine, vitamin B6, and folic acid between the cohorts. These results suggest a possible influence of the gut microbiome on the gut-brain axis and further on the incidence of ADHD symptoms.

The Dutch group (Szopinska-Tokov et al., 2020) increased the sample of Aarts et al. (2017). Although they used the same method as before (16S RNA sequencing), they did not show any differences in alpha diversity this time. In turn, they observed a smaller beta (between-sample diversity) in patients with ADHD, which significantly correlated with the severity of symptoms. At the genus level, ten were distinguished, which were characteristic of ADHD cases (bacteria belonging to the order Clostridiales were increased in ADHD, and the genus *Haemophilus* was reduced). Two genera were identified from this, the presence of which correlated with the severity of attention deficit symptoms (*Ruminococcaceae_UCG_004* and *Ruminococcus_2*). The authors suggest that the *Ruminococcaceae* family may be associated with neurobiological processes and ADHD. It may be of special interest that a larger number of *Ruminococcaceae_UCG_004* was also associated with the observed higher level of anxiety in mice after colonizing their intestines with microbial flora from patients with ADHD (Tengeler et al., 2020).

In the study of Szopinska-Tokov et al. (2020), no such correlations were found for symptoms of hyperactivity and impulsiveness. There was also no evidence of drug effects on the beta diversity or on the relationship between the amount of *Ruminococcaceae_UCG_004* and the severity of attention deficit. However, a decrease in *Phascolarctobacterium* and an increase in *Dialister* was observed in pharmacologically treated subjects.

3.2. Consideration and future perspectives

The results have differed significantly between researchers. For example, in Dutch studies (Aarts et al., 2017; Szopinska-Tokov et al., 2020), the majority were Firmicutes, while in Taiwan (Wang et al., 2020), the majority were Bacteroidetes. Other conclusions were also drawn about the diversity between the ADHD groups and the control group. Alpha diversity was calculated in 7 studies. No significant difference was found in 5 of them. Other studies have shown contradictory results (Prehn-Kristensen et al., 2018; Wang et al., 2020). Beta diversity in two out of four studies was not significant between groups (Wang et al., 2020; Stevens et al. 2019).

Three studies have observed *Bifidobacterium* as having a potential effect on ADHD (Pärtty et al., 2015; Aarts et al., 2017; Stevens et al., 2019). Their results were also contradictory - a reduction at 3 and 6 months of age in Pärtty et al. (2015) with no significant changes at age of 13, an increase in Aarts et al. (2017) and a decrease in Stevens et al. (2019). Aarts explains his results with a more accurate method, a larger research group, or differences in procedures or between groups. He also proposes a hypothesis about the later maturation of the gut microbiome in patients with ADHD.

In 4 studies, significant differences concerned the genus Bacteroides - Pärtty et al. (2015) observed a decrease in *Bacteroides* at 18 months of age and no difference at 13 years of age, and in 3 subsequent studies (Prehn-Kristensen et al., 2018; Wang et al., 2020; Wan et al., 2020) the authors consistently demonstrated an increase in *Bacteroides* and ADHD. *B. ovatus* and *OTU_7* (*Bacteroides* spec.) were correlated with the severity of ADHD symptoms (Prehn-Kristensen et al., 2018; Wang et al., 2020).

Finally, each team proposed different bacterial groups as potential new markers of ADHD - *Bifidobacterium* (Aarts et al., 2017; Stevens et al., 2019), *Neisseria* and *Bacteroides* (Prehn-Kristensen et al., 2018), *Faecalibacterium* (Jiang et al., 2018), *Bacteroides uniformis*, *Bacteroides ovatus* and *Sutterella stercoricanis* (Wang et al., 2020), *Ruminococcaceae* (Szopinska-Tokov et al., 2020). It is also likely that not one but different types of bacteria may be potentially associated with ADHD, and the gut microbiome is one of the many contributing factors in the etiology of ADHD.

There can be several reasons for the different results. A very significant limitation of research is the fact that research groups were usually small. Fecal samples of all the above-described analyzes were collected from only 209 participants. However, according to Stevens et al. (2019) power analysis indicates that the sample size in a single microbiota profiling study should be 200 participants.

The study groups also differed regarding the age of participants, from infants (Pärtty et al., 2015) to adulthood (Aarts et al., 2017; Szopinska-Tokov et al., 2020), while the composition of the intestinal microbiome and bacterial diversity are quite dynamic and change with age (Ottman et al., 2012; Yatsunenko et al., 2012; Arboleya et al., 2016; Odamaki et al., 2016).

The researches were conducted in various countries and climate zones. As is known, different cultures differ in nutrition patterns. In turn, the impact of residence and diet are obvious factors affecting the composition of the human intestinal microflora (Yatsunenko et al., 2012; Conlon and Bird, 2014). Despite this, only a few studies have considered dietary patterns.

It is worth noting that only two studies clearly indicated that they excluded gastrointestinal diseases from the cohorts (Jiang et al., 2018; Wan et al., 2020), despite the known effects of chronic bowel disease on intestinal dysbiosis (DuPont and DuPont, 2011). Wan et al. (2020) showed a greater susceptibility to constipation in the ADHD group. It is possible that the impulsivity in this group is the reason for reaching for highly processed constipating products, causing intestinal dysbiosis, or perhaps intestinal dysbiosis is responsible for the coexistence of constipation in patients with ADHD. The current research results do not allow to clearly indicate the solution, but it seems necessary for future studies of the ADHD microbiome to take into account the presented dependencies.

Antibiotics (Sullivan et al., 2001) and other drug use is also not indifferent to the composition of the microbiome. However, not all

studies included this criterion as an exclusion criterion. Stevens' study proved that micronutrient supplementation also influences the abundance of species in the gut microbiome. One of the studies showed that ADHD medication can also affect changes in the microbiome (Szopinska-Tokov et al., 2020). In the same study, there was no significant drug effect on beta diversity. According to Prehn-Kristensen et al. (2018) also had no effect on alpha diversity. However, these conclusions were drawn on the basis of only 4 treated patients. There were also not always clear criteria for exclusion from microbiome research. The authors did not always report the time that elapsed from taking antibiotics or other medication before taking the sample. Numerous factors, such as the type of delivery, prematurity, and breastfeeding, were not taken into account by the researchers, despite studies on their significant impact on the development of microbiota (Bokulich et al., 2016; Yassour et al., 2016).

After selecting the appropriate size and characteristics of the studied cohorts, it is time to collect samples, transport them, and then extract DNA material, sequencing and processing the results. Table 1 clearly shows the diversity between studies in this area. It is worth mentioning the various methods of profiling the microbiome that were used by researchers. With the development of science and technology in recent years, the possibilities of the methods used have increased. Each of them has its pros and cons. Unfortunately, the results are not always comparable. During the 16S rRNA gene sequencing and shotgun metagenomics comparison, it was shown that these two approaches give significantly different bacterial community structures of the same samples (Shah et al., 2010).

Within one method of sequencing, there are also many variants of the procedure. There are many protocols available for testing stool samples, published by groups such as the Human Microbiome Project, MetaHIT, the Earth Microbiome Project. Even if 16S RNA sequencing was used in several studies, the results may vary significantly. This is the result of discrepancies and errors at each stage of the analysis - sampling conditions, storage temperature, transport time, sample processing, primer selection, amplification, sequencing, bioinformatic analysis of the obtained results. There have been numerous publications describing in detail the problem of the lack of repeatability of biomedical research (Wintzingerode et al., 1997; Kunin et al., 2010; Goedert, 2013; Walker et al., 2015; Hiergeist et al., 2016; Lim et al., 2018). The significant impact of kit and laboratory contaminants has also been reported in the literature (Salter et al., 2014). The authors unanimously emphasize the need to standardize the methods and the possibility of their comparison. For this purpose, projects like the International Human Microbiome Standards (IHMS) and the MicroBiome Quality Control (MBQC) have been created. In their work, they compare the available research protocols, determine the factors most influencing the variability of the results and the strength of their impact at each stage of the research (Costea et al., 2017; Sinha et al., 2017; Greathouse et al., 2019). The multi-center activity of consortia is aimed at standardizing the protocols, updating them in line with the latest knowledge and ensuring the possibility of fine-tuning them to the specific characteristics of the study and obtaining optimally comparable results. After analyzing the presented results, the hypothesis concerning the influence of the gut microbiome on ADHD symptoms requires deepening and continuing research.

In light of the above considerations, it seems necessary to pay attention of future researchers to important methodological issues. One of the most important points is to conduct longitudinal tests on larger groups. Multidisciplinary cooperation will be useful in terms of selecting an appropriate sample. A child and adolescent psychiatrist is needed to diagnose and assess the severity of symptoms at the time of examination, and to exclude comorbidities such as ASD. Bearing in mind the changes in the microbiota in inflammatory bowel diseases, it is also important for a gastroenterologist to assess the presence of symptoms from the digestive system. Many confounding factors must be taken into account by carefully collecting medical history and questionnaires on diet, medication (including ADHD medication), antibiotic, supplement,

weight, and comorbidities. We cannot forget about the questions about the perinatal and early childhood period. Taking into account the variability of the microbiome over time, longitudinal studies with repeated measurements at different ages seem to be the best. Groups should be age-appropriate by covering a narrow age range. Due to the rapidly developing field of research on the human microbiome, future researchers should use current protocols to standardize the methodology, correctness of collecting, storing, sequencing and interpreting data. Attention is also paid to the detailed reporting of their research in terms of the characteristics of the studied groups and the methodology used. This will certainly make it easier for readers and future researchers to compare the obtained data. It should be noted that due to such a large variety of studies, no meta-analysis has yet appeared that could reliably synthesize the obtained results quantitatively and draw unequivocal conclusions.

After analyzing the research on the differences in the gut microbiota in patients with ADHD, another topic that is worth looking at for helping patients reduce symptoms and also for future research is the impact of using probiotic.

4. Probiotic supplementation and ADHD

4.1. Research review

Recently, many researchers have been interested in the influence of probiotic supplementation on mental health. Clinical studies have reported that probiotics could be beneficial in treating depression (Eltokhi et al., 2020; Wallace and Milev, 2017), anxiety (Eltokhi et al., 2020; Ng et al., 2018), and stress (Kato-Kataoka et al., 2016). We will present research on the impact of probiotic supplementation on patients' neurodevelopmental outcomes, which may be related to the later appearance of ADHD symptoms. We will also present research on the correlation of probiotic supplementation with the occurrence of ADHD symptoms and a study on the effect of probiotic supplementation on the health-related quality of life of children with ADHD diagnosis. The table below summarizes selected randomized trials (Table 2).

The influence of probiotic supplementation may be direct or indirect. Children with ADHD obtain higher scores on the gastrointestinal severity index and report constipation and flatulence more often than healthy children (Ming et al., 2018). Reducing these symptoms leads to the improvement in the quality of life for patients (Eltokhi et al., 2020). This can be achieved by changing the composition of the microbiota, for example, by probiotic supplementation. Unfortunately, in a systematic review of randomized trials, as described by Rianda et al. (2019), only 1 out of 7 studies showed a positive effect of probiotic supplementation on cognitive function.

Some studies demonstrated that *Lactobacillus rhamnosus* GG leads to stabilization of the gut permeability barrier, probably secondary to changes in tight junctions, mucin production and antigen-specific immunoglobulin A production (Pärtty et al., 2015; Isolauri et al., 2008). Moreover, the impact of *Lactobacillus rhamnosus* on the regulation of emotional behavior and on the central GABAergic system via the vagus nerve is associated with a number of neuropsychiatric disorders (Pärtty et al., 2015; Bravo et al., 2011; Enticott et al., 2010).

The results of the studies were evaluated using different methods.

One of the mentioned studies shows that *Lactobacillus rhamnosus* GG probiotic supplementation may lead to a reduction in the risk of developing ADHD. This demonstrates that probiotic supplementation could have a positive effect on cognitive function (Rianda et al., 2019); however, in most of the studies, follow-up ended before the age of 5, which is early for ADHD diagnosis. It is very probable that the supplementation effect declines over time because of the notable implications of environmental factors during child development. To obtain more comprehensive results, follow-up studies should have various observation time points.

A very interesting pilot randomized controlled trial carried out by

Table 2
Characteristics of included studies

Study	Aim of the study	Participants	Duration	Diagnostic instrument	Full name of strain	Form of probiotic	Daily dose in CFU/day	Results
Pärtty et al. (2015)	evaluation of of the impact of the gut brain-axis on the occurrence of ADHD and AS	75 children	first six months of life, mothers had taken supplementation for 4 weeks before delivery and continued to take it for 6 months after delivery if they breastfeed, gut microbiota was evaluated at the age of 3 weeks, 3, 6, 12, 18, 24 months, follow up - 13 years old	attention deficit hyperactivity disorder (ADHD) and Asperger syndrome (AS) - ICD-10	*Lactobacillus rhamnosus* GG	capsule, capsule contents	10^10 CFU daily	after reaching 13 y.o. in the placebo group 3 children out of 35 were diagnosed with ADHD (8,6%), 1 child out of 35 was diagnosed with AS (2,8%), 2 children out of 35 was diagnosed with both ADHD and AS (5,7%)
Slykerman et al. (2018)	to assess if probiotic supplementation in the early stages of life enhances neurocognitive outcomes evaluated at 11 years old	474 children	35 weeks gestation until 6 months if breastfeeding, and the supplementation for infants from birth to 2 years, evaluation was performed at 3, 6, 12, 18 months and 2, 4, 6 years old, follow up - 11 years old	The Wechsler Intelligence Scale for Children—Fourth Edition (WISC-IV), Behavior Rating Inventory of Executive Function (BRIEF), Conners 3rd Edition™ (Conners 3™), The Strengths and Difficulties Questionnaire (SDQ), Multidimensional Anxiety Scale for Children 2nd Ed (MASC 2), The Centre for Epidemiological Studies Depression Scale for Children (CES-DC), The Conners Continuous Performance Test 3rd Edition™ (Conners CPT 3™), Cambridge Neuropsychological Test Automated Battery (CANTAB)	*Lactobacillus rhamnosus* HN001, *Bifidobacterium animalis* subsp. *lactis* HN019	capsule, powder mixed with water, breast milk or formula or with food	*Lactobacillus rhamnosus* strain HN001–6 × 10^9 CFU daily, *Bifidobacterium animalis* subsp. *lactis* HN019 - 9 × 10^9 CFU daily	342 participants were assessed, but no important differences were observed in the neurocognitive outcomes between the groups
Akar et al. (2016)	assessment of the neurodevelopment results of very low birth weight (VLBW) preterm infants who were given probiotic supplementation to prevent necrotizing enterocolitis (NEC)	400 children	very low birth weight (VLBW) preterm infants gestational age ≤ 32 weeks or birth weight ≤ 1500 g feeded enterally, supplementation - first feed until discharge, follow up - extensive evaluation was performed at 18–24 months' corrected age	Bayley Scales of Infant Development II (BSID-II), sensory and neurological performance was assessed using standard techniques	*Lactobacillus reuteri*	no data	100 million organisms	370 participants - follow up, no substantial differences in any of the neurodevelopmental and sensory outcomes among the 2 groups
Chou et al. (2010)	to measure growth and neurodevelopmental outcomes in preterm very low birth weight (PVLBW) infants supplemented with oral probiotics to prevent necrotizing enterocolitis (NEC)	367 children	infants feeded enterally that lived more than 7 days after birth, supplementation until discharge, follow up - participants were observed every 6 months up to 3 years corrected age	Bayley Scales of Infant Development II (BSID-II), sensory and neurological performance was assessed using standard techniques	Lactobacillus acidophilus + Bifidobacteria infantis	probiotic with breast milk	10^9 CFU twice daily - *Lactobacillus acidophilus* + 10^9 CFU twice daily - *Bifidobacteria infantis*	no substantial differences in growth or in any of the neurodevelopmental and sensory outcomes among the two groups
Firmansyah et al. (2011)	comparison of the effect of supplementation of milk including synbiotics and LCPUFA or a control milk on the growth and development of toddlers	393 toddlers	screening took place every two months starting at the age of 12 months until the age of 24 months	Bayley Scale for Infant and Toddler Development (BSID III), Cochran- Mantel-Haenszel test	*Bifidobacterium longum* (BL999), *Lactobacillus rhamonosus* (LPR), prebiotics (inulin and fructo-oligosaccharides), and long-chain	milk containing synbiotics	57,6 × 10^7 CFU daily - *Bifidobacterium longum* + 115,2 × 10^7 CFU	no substantial differences in the mental and motor developmental indicators among the two groups, regarding cognitive and adaptive behavior scores non

Table 2 (*continued*)

Study	Aim of the study	Participants	Duration	Diagnostic instrument	Full name of strain	Form of probiotic	Daily dose in CFU/day	Results
					polyunsaturated fatty acids (LCPUFA)		daily - *Lactobacillus rhamnosus*	substantial differences were observed - between 12 and 16 months, the results were slightly higher in synbiotic group in comparison to control group
Kumperscak et al. (2020)	evaluate the possible impact of the probiotic on core symptoms, health-related quality of life (QoL), and serum levels of cytokines in children and adolescents with ADHD	32 children and adolescents 4–17 years old	assessment of participants was performed twice – at the start of the trial and after 3 months	ADHD Parent-Report Rating Scale-IV: Home Version; the Child Self-Report and Parent Proxy-Report of the Pediatric Quality of Life InventoryTM (PedsQLTM) 4.0 Generic Core Scale; the Parent Form (CBCL/6–18) and the Teacher Report Form (TRF) of the Child Behavior Checklist (CBCL) for ages 6–18 of the Achenbach System of Empirically Based Assessment (ASEBA), serum levels of cytokines	*Lactobacillus rhamnosus* GG	capsule	10ˆ10 CFU daily	significant improvement in the PedsQL Child Self – Report Total score in the probiotic group but not in the placebo group, but no differences in core symptoms or emotional and behavioural problems were noted between the groups, pro-inflammatory cytokines - ↓ in the probiotic group in terms of the serum levels of IL-6, IL-12 p70, and TNF-α, of which IL-12 p70 and TNF- only in the probiotic group, serum level of IL-6 was also ↓ in the placebo group

Kumperscak et al. (2020) was conducted in a group of 32 drug-naive children and adolescents aged 4 to 17 years to evaluate the possible impact of the probiotic on core symptoms, health-related quality of life (QoL), and serum levels of cytokines in children and adolescents with ADHD. One group of participants was given capsules with the probiotic strain *Lactobacillus rhamnosus* GG (10^{10} CFU daily). The rest of the participants received placebo. Assessment of participants was performed twice – at the start of the trial and after 3 months. The study covered laboratory serum analysis for the following cytokines: IL-1B, IL-2R, IL-4, IL-6, IL-8, IL-10, IL-12 p70, IL-17, IL-18, and TNF-α. The authors observed a significant improvement in the PedsQL Child Self – Report Total score in the probiotic group but not in the placebo group, but no differences in core symptoms or emotional and behavioural problems were noted between the groups. Pro-inflammatory cytokines showed a decrease in the probiotic group in terms of the serum levels of IL-6, IL-12 p70, and TNF-α, of which IL-12 p70 and TNF-α decreased only in the probiotic group. The serum level of IL-6 was also reduced in the placebo group.

The discussed research broadens our knowledge of the correlation between the supply of probiotic and the occurrence of ADHD symptoms. The presented research shows only to some extent the relationship between probiotic supplementation and ADHD symptoms. Unfortunately are still uconclusive and there is still impossible to frame any clinical recommendation or guidelines. Subsequent research should be extended taking into account the aspects presented in the next part of the article.

4.2. Consideration and future perspectives

Some of the issues regarding the influence of probiotic supplementation on cognitive function require additional examination. Currently, it is unclear which probiotic strain affects cognition. Trials in the future should take into consideration the methods of cognitive evaluation with emphasis on innovative approaches for assessing earlier brain development, such as foetal brain imaging (Rianda et al., 2019; Griffiths et al., 2017).

A future study should include people diagnosed with ADHD who will be taking probiotic supplementation, be multicenter, affect a larger group of patients, in different age groups, have a duration time of several months (all these elements together were not present in the discussed studies). When designing a future study, attention should be paid to the storage of probiotic preparations in appropriate conditions, study participants' diet, qualitative and quantitative studies on the gut microbiota, the use of several different scales, questionnaires as well as the possibility of using smartwatches and mobile applications to assess changes in the severity of symptoms. There is an ongoing study that has been designed to cover many of the aspects outlined above.

On February 2019 in Frankfurt, Barcelona and Budapest started an interesting multicentre, double-blind, randomized controlled trial, which is planned to last two years, involving 180 participants with a diagnosis of attention deficit/hyperactivity disorder (ADHD) (and/or borderline personality disorder), at the age of 18–65. In the trial participants are taking synbiotic formula (probiotic + prebiotic - Synbiotic 2000 Forte 400 (SF) containing 100 billion of each of *Pediococcus pentosaceus* 5–33:3, *Lactobacillus paracasei* subsp. *paracasei* 19, *Lactobacillus plantarum* 2362, and *Leuconostoc mesenteroides* 77:1, with four bioactive fermentable fibers (2.5 g each of β-glucan, inulin, pectin, and resistant starch) or placebo. Changes in scope of „general psychopathology, ADHD symptoms, neurocognitive function, somatic parameters, physical activity, nutritional intake, and health-related quality of life" will be assessed (Arteaga-Henríquez et al., 2020).

5. Conclusions and future directions

The aim of the study was to systematize the current knowledge as part of the review of the latest research on the microbiota, the supply of probiotics and collations with the presence of ADHD symptoms, taking

into account the neurophysiological basis.

In the research presented in this article on the correlation of the composition of the microbiota with the occurrence of ADHD symptoms, no unambiguous outcomes were obtained. In further studies on the composition of the microbiota in ADHD and the correlation with the occurrence of symptoms, it is worth paying attention to the unification of research methods and comparing the results in patients in a similar age group. To obtain more representative results, it is necessary to perform tests involving a larger group of patients.

In this article, we wanted to present the relationship between the supply of probiotic and: 1) the occurrence of ADHD symptoms,

2) the influence on patients' neurodevelopmental outcomes,

3) the impact on the health-related quality of life of children with ADHD diagnosis.

The results are varied. Regarding supplementation with the probiotic *Lactobacillus rhamnosus* GG strain, one study has shown that it has a beneficial effect on cognitive function and, consequently, the occurrence of ADHD symptoms. In another study, it has been demonstrated to have an advantageous effect on the health-related quality of life described by children and adolescents. Taking this information into consideration, the current evidence is insufficient to recommend treating ADHD with probiotic supplementation.

To assess the subject more accurately, further studies are needed that last longer and involve a larger group of patients, using different assessment methods such as in vivo observation, actigraphy or the use of digital methods. The studies should be conducted in various age groups lasting for at least several months. The multicentre study presented in the future perspectives section seems to be promising in terms of the correlation between the supply of synbiotic preparations and the occurrence of ADHD symptoms. The presented overview of the research and its results constitute a contribution to science and knowledge on the topic chosen and described by us. In our work, we wanted to include information on how future research should be designed to obtain the most accurate results.

Declaration of Competing Interest

None.

References

Aarts, E., Ederveen, T.H.A., Naaijen, J., Zwiers, M.P., Boekhorst, J., Timmerman, H.M., Smeekens, S.P., Netea, M.G., Buitelaar, J.K., Franke, B., van Hijum, S.A.F.T., Arias Vasquez, A., 2017. Gut microbiome in ADHD and its relation to neural reward anticipation. PLoS One 12, e0183509. https://doi.org/10.1371/journal.pone.0183509.

Akar, M., Eras, Z., Oncel, M.Y., Arayici, S., Guzoglu, N., Canpolat, F.E., Uras, N., Oguz, S. S., 2016. Impact of oral probiotics on neurodevelopmental outcomes in preterm infants. J. Matern. Neonatal Med. 30, 411–415. https://doi.org/10.1080/14767058.2016.1174683.

Arboleya, S., Watkins, C., Stanton, C., Ross, R.P., 2016. Gut Bifidobacteria populations in human health and aging. Front. Microbiol. 7, 1204. https://doi.org/10.3389/fmicb.2016.01204.

Arteaga-Henríquez, G., Rosales-Ortiz, S.K., Arias-Vásquez, A., Bitter, I., Ginsberg, Y., Ibañez-Jimenez, P., Kilencz, T., Lavebratt, C., Matura, S., Reif, A., Rethelyi, J., Richarte, V., Rommelse, N., Siegl, A., Ramos-Quiroga, J.A., 2020. Treating impulsivity with probiotics in adults (PROBIA): study protocol of a multicenter, double-blind, randomized, placebo-controlled trial. Trials 21, 161. https://doi.org/10.1186/s13063-019-4040-x.

Asano, Y., Hiramoto, T., Nishino, R., Aiba, Y., Kimura, T., Yoshihara, K., Koga, Y., Sudo, N., 2012. Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. Am. J. Physiol. Liver Physiol. 303, G1288–G1295. https://doi.org/10.1152/ajpgi.00341.2012.

Bercik, P., Denou, E., Collins, J., Jackson, W., Lu, J., Jury, J., Deng, Y., Blennerhassett, P., Macri, J., McCoy, K.D., Verdu, E.F., Collins, S.M., 2011. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 141, 599–609 e3. https://doi.org/10.1053/j.gastro.2011.04.052.

Berridge, C.W., Stalnaker, T.A., 2002. Relationship between low-dose amphetamine-induced arousal and extracellular norepinephrine and dopamine levels within prefrontal cortex. Synapse 46, 140–149. https://doi.org/10.1002/syn.10131.

Bokulich, N.A., Chung, J., Battaglia, T., Henderson, N., Jay, M., Li, H., Lieber, D., Wu, F., Perez-Perez, G.I., Chen, Y., Schweizer, W., Zheng, X., Contreras, M., Dominguez-Bello, M.G., Blaser, M.J., 2016. Antibiotics, birth mode, and diet shape microbiome maturation during early life. Sci. Transl. Med. 8 https://doi.org/10.1126/scitranslmed.aad7121.

Bonaz, B., Bazin, T., Pellissier, S., 2018. The vagus nerve at the interface of the microbiota-gut-brain axis. Front. Neurosci. 12 https://doi.org/10.3389/fnins.2018.00049.

Bonvicini, C., Cortese, S., Maj, C., Baune, B.T., Faraone, S.V., Scassellati, C., 2020. DRD4 48 bp multiallelic variants as age-population-specific biomarkers in attention-deficit/hyperactivity disorder. Transl. Psychiatry 10, 70. https://doi.org/10.1038/s41398-020-0755-4.

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., Cryan, J.F., 2011. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. 108, 16050–16055. https://doi.org/10.1073/pnas.1102999108.

Byrne, E.M., Zhu, Z., Qi, T., Skene, N.G., Bryois, J., Pardinas, A.F., Stahl, E., Smoller, J. W., Rietschel, M., Owen, M.J., Walters, J.T.R., O'Donovan, M.C., McGrath, J.G., Hjerling-Leffler, J., Sullivan, P.F., Goddard, M.E., Visscher, P.M., Yang, J., Wray, N. R., 2020. Conditional GWAS analysis to identify disorder-specific SNPs for psychiatric disorders. Mol. Psychiatry. https://doi.org/10.1038/s41380-020-0705-9.

Cheng, S., Han, B., Ding, M., Wen, Y., Ma, M., Zhang, L., Qi, X., Cheng, B., Li, P., Kafle, O. P., Liang, X., Liu, L., Du, Y., Zhao, Y., Zhang, F., 2020. Identifying psychiatric disorder-associated gut microbiota using microbiota-related gene set enrichment analysis. Brief. Bioinform. 21, 1016–1022. https://doi.org/10.1093/bib/bbz034.

Chou, I.-C., Kuo, H.-T., Chang, J.-S., Wu, S.-F., Chiu, H.-Y., Su, B.-H., Lin, H.-C., 2010. Lack of effects of oral probiotics on growth and neurodevelopmental outcomes in preterm very low birth weight infants. J. Pediatr. 156, 393–396. https://doi.org/10.1016/j.jpeds.2009.09.051.

Conlon, M., Bird, A., 2014. The impact of diet and lifestyle on gut microbiota and human health. Nutrients 7, 17–44. https://doi.org/10.3390/nu7010017.

Cortese, S., 2012. The neurobiology and genetics of attention-deficit/hyperactivity disorder (ADHD): what every clinician should know. Eur. J. Paediatr. Neurol. 16, 422–433. https://doi.org/10.1016/j.ejpn.2012.01.009.

Costea, P.I., Zeller, G., Sunagawa, S., Pelletier, E., Alberti, A., Levenez, F., Tramontano, M., Driessen, M., Hercog, R., Jung, F.-E., Kultima, J.R., Hayward, M.R., Coelho, L.P., Allen-Vercoe, E., Bertrand, L., Blaut, M., Brown, J.R.M., Carton, T., Cools-Portier, S., Daigneault, M., Derrien, M., Druesne, A., de Vos, W.M., Finlay, B. B., Flint, H.J., Guarner, F., Hattori, M., Heilig, H., Luna, R.A., van Hylckama Vlieg, J., Junick, J., Klymiuk, I., Langella, P., Le Chatelier, E., Mai, V., Manichanh, C., Martin, J.C., Mery, C., Morita, H., O'Toole, P.W., Orvain, C., Patil, K. R., Penders, J., Persson, S., Pons, N., Popova, M., Salonen, A., Saulnier, D., Scott, K. P., Singh, B., Slezak, K., Veiga, P., Versalovic, J., Zhao, L., Zoetendal, E.G., Ehrlich, S. D., Dore, J., Bork, P., 2017. Towards standards for human fecal sample processing in metagenomic studies. Nat. Biotechnol. 35, 1069–1076. https://doi.org/10.1038/nbt.3960.

de Araujo, I.E., Ferreira, J.G., Tellez, L.A., Ren, X., Yeckel, C.W., 2012. The gut–brain dopamine axis: a regulatory system for caloric intake. Physiol. Behav. 106, 394–399. https://doi.org/10.1016/j.physbeh.2012.02.026.

Demontis, D., Walters, R.K., Martin, J., Mattheisen, M., Als, T.D., Agerbo, E., Baldursson, G., Belliveau, R., Bybjerg-Grauholm, J., Bækvad-Hansen, M., Cerrato, F., Chambert, K., Churchhouse, C., Dumont, A., Eriksson, N., Gandal, M., Goldstein, J.I., Grasby, K.L., Grove, J., Gudmundsson, O.O., Hansen, C.S., Hauberg, M.E., Hollegaard, M.V., Howrigan, D.P., Huang, H., Maller, J.B., Martin, A.R., Martin, N. G., Moran, J., Pallesen, J., Palmer, D.S., Pedersen, C.B., Pedersen, M.G., Poterba, T., Poulsen, J.B., Ripke, S., Robinson, E.B., Satterstrom, F.K., Stefansson, H., Stevens, C., Turley, P., Walters, G.B., Won, H., Wright, M.J., Andreassen, O.A., Asherson, P., Burton, C.L., Boomsma, D.I., Cormand, B., Dalsgaard, S., Franke, B., Gelernter, J., Geschwind, D., Hakonarson, H., Haavik, J., Kranzler, H.R., Kuntsi, J., Langley, K., Lesch, K.-P., Middeldorp, C., Reif, A., Rohde, L.A., Roussos, P., Schachar, R., Sklar, P., Sonuga-Barke, E.J.S., Sullivan, P.F., Thapar, A., Tung, J.Y., Waldman, I.D., Medland, S.E., Stefansson, K., Nordentoft, M., Hougaard, D.M., Werge, T., Mors, O., Mortensen, P.B., Daly, M.J., Faraone, S.V., Børglum, A.D., Neale, B.M., 2019. Discovery of the first genome-wide significant risk loci for attention deficit/hyperactivity disorder. Nat. Genet. 51, 63–75. https://doi.org/10.1038/s41588-018-0269-7.

Didari, T., Mozaffari, S., Nikfar, S., Abdollahi, M., 2015. Effectiveness of probiotics in irritable bowel syndrome: updated systematic review with meta-analysis. World J. Gastroenterol. 21, 3072–3084. https://doi.org/10.3748/wjg.v21.i10.3072.

Dinan, T.G., Cryan, J.F., 2015. The impact of gut microbiota on brain and behaviour. Curr. Opin. Clin. Nutr. Metab. Care 18, 552–558. https://doi.org/10.1097/MCO.0000000000000221.

DuPont, A.W., DuPont, H.L., 2011. The intestinal microbiota and chronic disorders of the gut. Nat. Rev. Gastroenterol. Hepatol. 8, 523–531. https://doi.org/10.1038/nrgastro.2011.133.

Eltokhi, A., Janmaat, I.E., Genedi, M., Haarman, B.C.M., Sommer, I.E.C., 2020. Dysregulation of synaptic pruning as a possible link between intestinal microbiota dysbiosis and neuropsychiatric disorders. J. Neurosci. Res. 98, 1335–1369. https://doi.org/10.1002/jnr.24616.

Enticott, P.G., Rinehart, N.J., Tonge, B.J., Bradshaw, J.L., Fitzgerald, P.B., 2010. A preliminary transcranial magnetic stimulation study of cortical inhibition and excitability in high-functioning autism and Asperger disorder. Dev. Med. Child Neurol. 52, e179–e183. https://doi.org/10.1111/j.1469-8749.2010.03665.x.

Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A., Sklar, P., 2005. Molecular genetics of attention-deficit/hyperactivity disorder. Biol. Psychiatry 57, 1313–1323. https://doi.org/10.1016/j.biopsych.2004.11.024.

Firmansyah, A., Dwipoerwantoro, P.G., Kadim, M., Alatas, S., Conus, N., Lestarina, L., Bouisset, F., Steenhout, P., 2011. Improved growth of toddlers fed a milk containing synbiotics. Asia Pac. J. Clin. Nutr. 20, 69–76.

Goedert, J.J., 2013. The microbiota and human health: beyond exploration. Eur. J. Clin. Investig. 43, 657–659. https://doi.org/10.1111/eci.12100.

Greathouse, K.L., Sinha, R., Vogtmann, E., 2019. DNA extraction for human microbiome studies: the issue of standardization. Genome Biol. 20, 212. https://doi.org/10.1186/s13059-019-1843-8.

Griffiths, P.D., Bradburn, M., Campbell, M.J., Cooper, C.L., Graham, R., Jarvis, D., Kilby, M.D., Mason, G., Mooney, C., Robson, S.C., Wailoo, A., 2017. Use of MRI in the diagnosis of fetal brain abnormalities in utero (MERIDIAN): a multicentre, prospective cohort study. Lancet 389, 538–546. https://doi.org/10.1016/S0140-6736(16)31723-8.

Grimm, O., Kittel-Schneider, S., Reif, A., 2018. Recent developments in the genetics of attention-deficit hyperactivity disorder. Psychiatry Clin. Neurosci. 72, 654–672. https://doi.org/10.1111/pcn.12673.

Grimm, O., Kranz, T.M., Reif, A., 2020. Genetics of ADHD: what should the clinician know? Curr. Psychiatry Rep. 22, 18. https://doi.org/10.1007/s11920-020-1141-x.

Heijtz, R.D., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., Hibberd, M.L., Forssberg, H., Pettersson, S., 2011. Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. 108, 3047–3052. https://doi.org/10.1073/pnas.1010529108.

Hiergeist, A., Reischl, U., Gessner, A., 2016. Multicenter quality assessment of 16S ribosomal DNA-sequencing for microbiome analyses reveals high inter-center variability. Int. J. Med. Microbiol. 306, 334–342. https://doi.org/10.1016/j.ijmm.2016.03.005.

Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow, J., Reisman, S.E., Petrosino, J.F., Patterson, P.H., Mazmanian, S.K., 2013. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155, 1451–1463. https://doi.org/10.1016/j.cell.2013.11.024.

Isolauri, E., Kalliomaki, M., Laitinen, K., Salminen, S., 2008. Modulation of the maturing gut barrier and microbiota: a novel target in allergic disease. Curr. Pharm. Des. 14, 1368–1375. https://doi.org/10.2174/138161208784480207.

Jiang, H., Zhou, Y., Zhou, G., Li, Y., Yuan, J., Li, X., Ruan, B., 2018. Gut microbiota profiles in treatment-naïve children with attention deficit hyperactivity disorder. Behav. Brain Res. 347, 408–413. https://doi.org/10.1016/j.bbr.2018.03.036.

Kato-Kataoka, A., Nishida, K., Takada, M., Kawai, M., Kikuchi-Hayakawa, H., Suda, K., Ishikawa, H., Gondo, Y., Shimizu, K., Matsuki, T., Kushiro, A., Hoshi, R., Watanabe, O., Igarashi, T., Miyazaki, K., Kuwano, Y., Rokutan, K., 2016. Fermented milk containing Lactobacillus casei strain Shirota preserves the diversity of the gut microbiota and relieves abdominal dysfunction in healthy medical students exposed to academic stress. Appl. Environ. Microbiol. 82, 3649–3658. https://doi.org/10.1128/AEM.04134-15.

Kelly, J.R., Borre, Y., Brien, C., Patterson, E., El Aidy, S., Deane, J., Kennedy, P.J., Beers, S., Scott, K., Moloney, G., Hoban, A.E., Scott, L., Fitzgerald, P., Ross, P., Stanton, C., Clarke, G., Cryan, J.F., Dinan, T.G., 2016. Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. J. Psychiatr. Res. 82, 109–118. https://doi.org/10.1016/j.jpsychires.2016.07.019.

Kumperscak, H.G., Gricar, A., Ülen, I., Micetic-Turk, D., 2020. A pilot randomized control trial with the probiotic strain Lactobacillus rhamnosus GG (LGG) in ADHD: children and adolescents report better health-related quality of life. Front. Psychiatry 11. https://doi.org/10.3389/fpsyt.2020.00181.

Kunin, V., Engelbrektson, A., Ochman, H., Hugenholtz, P., 2010. Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates. Environ. Microbiol. 12, 118–123. https://doi.org/10.1111/j.1462-2920.2009.02051.x.

Lim, M.Y., Song, E.-J., Kim, S.H., Lee, J., Nam, Y.-D., 2018. Comparison of DNA extraction methods for human gut microbial community profiling. Syst. Appl. Microbiol. 41, 151–157. https://doi.org/10.1016/j.syapm.2017.11.008.

Lyte, M., 2016. Microbial Endocrinology: Interkingdom Signaling in Infectious Disease and Health, Advances in Experimental Medicine and Biology. Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-20215-0.

Ma, C.-L., Arnsten, A.F.T., Li, B.-M., 2005. Locomotor hyperactivity induced by blockade of prefrontal cortical α2-adrenoceptors in monkeys. Biol. Psychiatry 57, 192–195. https://doi.org/10.1016/j.biopsych.2004.11.004.

Martin, C.R., Osadchiy, V., Kalani, A., Mayer, E.A., 2018. The brain-gut-microbiome axis. Cell. Mol. Gastroenterol. Hepatol. 6, 133–148. https://doi.org/10.1016/j.jcmgh.2018.04.003.

Mathee, K., Cickovski, T., Deoraj, A., Stollstorff, M., Narasimhan, G., 2020. The gut microbiome and neuropsychiatric disorders: implications for attention deficit hyperactivity disorder (ADHD). J. Med. Microbiol. 69, 14–24. https://doi.org/10.1099/jmm.0.001112.

Ming, X., Chen, N., Ray, C., Brewer, G., Kornitzer, J., Steer, R.A., 2018. A gut feeling: a hypothesis of the role of the microbiome in attention-deficit/hyperactivity disorders. Child Neurol. Open 5. https://doi.org/10.1177/2329048X18786799, 2329048X1878679.

Minter, M.R., Zhang, C., Leone, V., Ringus, D.L., Zhang, X., Oyler-Castrillo, P., Musch, M.W., Liao, F., Ward, J.F., Holtzman, D.M., Chang, E.B., Tanzi, R.E., Sisodia, S.S., 2016. Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. Sci. Rep. 6, 30028. https://doi.org/10.1038/srep30028.

Ng, Q.X., Peters, C., Ho, C.Y.X., Lim, D.Y., Yeo, W.-S., 2018. A meta-analysis of the use of probiotics to alleviate depressive symptoms. J. Affect. Disord. 228, 13–19. https://doi.org/10.1016/j.jad.2017.11.063.

Odamaki, T., Kato, K., Sugahara, H., Hashikura, N., Takahashi, S., Xiao, J., Abe, F., Osawa, R., 2016. Age-related changes in gut microbiota composition from newborn to centenarian: a cross-sectional study. BMC Microbiol. 16, 90. https://doi.org/10.1186/s12866-016-0708-5.

Ottman, N., Smidt, H., de Vos, W.M., Belzer, C., 2012. The function of our microbiota: who is out there and what do they do? Front. Cell. Infect. Microbiol. 2 https://doi.org/10.3389/fcimb.2012.00104, 104-undefined.

Pärtty, A., Kalliomäki, M., Wacklin, P., Salminen, S., Isolauri, E., 2015. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. Pediatr. Res. 77, 823–828. https://doi.org/10.1038/pr.2015.51.

Petra, A.I., Panagiotidou, S., Hatziagelaki, E., Stewart, J.M., Conti, P., Theoharides, T.C., 2015. Gut-microbiota-brain Axis and its effect on neuropsychiatric disorders with suspected immune dysregulation. Clin. Ther. 37, 984–995. https://doi.org/10.1016/j.clinthera.2015.04.002.

Prehn-Kristensen, A., Zimmermann, A., Tittmann, L., Lieb, W., Schreiber, S., Baving, L., Fischer, A., 2018. Reduced microbiome alpha diversity in young patients with ADHD. PLoS One 13, e0200728. https://doi.org/10.1371/journal.pone.0200728.

Prince, J., 2008. Catecholamine dysfunction in attention-deficit/hyperactivity disorder. J. Clin. Psychopharmacol. 28, S39–S45. https://doi.org/10.1097/JCP.0b013e318174f92a.

Rianda, D., Agustina, R., Setiawan, E.A., Manikam, N.R.M., 2019. Effect of probiotic supplementation on cognitive function in children and adolescents: a systematic review of randomised trials. Benefic. Microbes 10, 873–882. https://doi.org/10.3920/BM2019.0068.

Salter, S.J., Cox, M.J., Turek, E.M., Calus, S.T., Cookson, W.O., Moffatt, M.F., Turner, P., Parkhill, J., Loman, N.J., Walker, A.W., 2014. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 12, 87. https://doi.org/10.1186/s12915-014-0087-z.

Sampson, T.R., Debelius, J.W., Thron, T., Janssen, S., Shastri, G.G., Ilhan, Z.E., Challis, C., Schretter, C.E., Rocha, S., Gradinaru, V., Chesselet, M.-F., Keshavarzian, A., Shannon, K.M., Krajmalnik-Brown, R., Wittung-Stafshede, P., Knight, R., Mazmanian, S.K., 2016. Gut microbiota regulate motor deficits and Neuroinflammation in a model of Parkinson's disease. Cell 167, 1469–1480 e12. https://doi.org/10.1016/j.cell.2016.11.018.

Sandgren, A.M., Brummer, R.J.M., 2018. ADHD-originating in the gut? The emergence of a new explanatory model. Med. Hypotheses 120, 135–145. https://doi.org/10.1016/j.mehy.2018.08.022.

Seyedabadi, M., Fakhfouri, G., Ramezani, V., Mehr, S.E., Rahimian, R., 2014. The role of serotonin in memory: interactions with neurotransmitters and downstream signaling. Exp. Brain Res. 232, 723–738. https://doi.org/10.1007/s00221-013-3818-4.

Shah, N., Tang, H., Doak, T.G., Ye, Y., 2010. Comparing bacterial communities inferred from 16S rRNA gene sequencing and shotgun metagenomics. Biocomputing 2011, 165–176. https://doi.org/10.1142/9789814335058_0018.

Sinha, R., Abu-Ali, G., Vogtmann, E., Fodor, A.A., Ren, B., Amir, A., Schwager, E., Crabtree, J., Ma, S., Abnet, C.C., Knight, R., White, O., Huttenhower, C., 2017. Assessment of variation in microbial community amplicon sequencing by the microbiome quality control (MBQC) project consortium. Nat. Biotechnol. 35, 1077–1086. https://doi.org/10.1038/nbt.3981.

Slykerman, R.F., Kang, J., Van Zyl, N., Barthow, C., Wickens, K., Stanley, T., Coomarasamy, C., Purdie, G., Murphy, R., Crane, J., Mitchell, E.A., 2018. Effect of early probiotic supplementation on childhood cognition, behaviour and mood a randomised, placebo-controlled trial. Acta Paediatr. 107, 2172–2178. https://doi.org/10.1111/apa.14590.

Spadoni, I., Zagato, E., Bertocchi, A., Paolinelli, R., Hot, E., Di Sabatino, A., Caprioli, F., Bottiglieri, L., Oldani, A., Viale, G., Penna, G., Dejana, E., Rescigno, M., 2015. A gut-vascular barrier controls the systemic dissemination of bacteria. Science 80 (350), 830–834. https://doi.org/10.1126/science.aad0135.

Stevens, A.J., Purcell, R.V., Darling, K.A., et al., 2019. Human gut microbiome changes during a 10 week Randomised Control Trial for micronutrient supplementation in children with attention deficit hyperactivity disorder. Sci Rep 9, 10128. https://doi.org/10.1038/s41598-019-46146-3.

Strandwitz, P., 2018. Neurotransmitter modulation by the gut microbiota. Brain Res. 1693, 128–133. https://doi.org/10.1016/j.brainres.2018.03.015.

Sullivan, Å., Edlund, C., Nord, C.E., 2001. Effect of antimicrobial agents on the ecological balance of human microflora. Lancet Infect. Dis. 1, 101–114. https://doi.org/10.1016/S1473-3099(01)00066-4.

Szopinska-Tokov, J., Dam, S., Naaijen, J., Konstanti, P., Rommelse, N., Belzer, C., Buitelaar, J., Franke, B., Aarts, E., Arias Vasquez, A., 2020. Investigating the gut microbiota composition of individuals with attention-deficit/hyperactivity disorder and association with symptoms. Microorganisms 8, 406. https://doi.org/10.3390/microorganisms8030406.

Tengeler, A.C., Dam, S.A., Wiesmann, M., Naaijen, J., van Bodegom, M., Belzer, C., Dederen, P.J., Verweij, V., Franke, B., Kozicz, T., Arias Vasquez, A., Kiliaan, A.J., 2020. Gut microbiota from persons with attention-deficit/hyperactivity disorder affects the brain in mice. Microbiome 8, 44. https://doi.org/10.1186/s40168-020-00816-x.

Thomas, R., Sanders, S., Doust, J., Beller, E., Glasziou, P., 2015. Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. Pediatrics 135, e994–e1001. https://doi.org/10.1542/peds.2014-3482.

Tripp, G., Wickens, J.R., 2009. Neurobiology of ADHD. Neuropharmacology 57, 579–589. https://doi.org/10.1016/j.neuropharm.2009.07.026.

Tsavkelova, E.A., Botvinko, I.V., Kudrin, V.S., Oleskin, A.V., 2000. Detection of neurotransmitter amines in microorganisms with the use of high-performance liquid chromatography. Dokl. Biochem. Proc. Acad. Sci. USSR Biochem. Sect. 372, 115.

Walker, A.W., Martin, J.C., Scott, P., Parkhill, J., Flint, H.J., Scott, K.P., 2015. 16S rRNA gene-based profiling of the human infant gut microbiota is strongly influenced by sample processing and PCR primer choice. Microbiome 3, 26. https://doi.org/10.1186/s40168-015-0087-4.

Wang, L.J., Yang, C.Y., Chou, W.J., et al., 2020. Gut microbiota and dietary patterns in children with attention-deficit/hyperactivity disorder. Eur Child Adolesc Psychiatry 29, 287–297. https://doi.org/10.1007/s00787-019-01352-2.

Wallace, C.J.K., Milev, R., 2017. The effects of probiotics on depressive symptoms in humans: a systematic review. Ann. General Psychiatry 16, 14. https://doi.org/10.1186/s12991-017-0138-2.

Wan, L., Ge, W.-R., Zhang, S., Sun, Y.-L., Wang, B., Yang, G., 2020. Case-control study of the effects of gut microbiota composition on neurotransmitter metabolic pathways in children with attention deficit hyperactivity disorder. Front. Neurosci. 14 https://doi.org/10.3389/fnins.2020.00127.

Wang, L.-J., Yang, C.-Y., Chou, W.-J., Lee, M.-J., Chou, M.-C., Kuo, H.-C., Yeh, Y.-M., Lee, S.-Y., Huang, L.-H., Li, S.-C., 2020. Gut microbiota and dietary patterns in children with attention-deficit/hyperactivity disorder. Eur. Child Adolesc. Psychiatry 29, 287–297. https://doi.org/10.1007/s00787-019-01352-2.

Wintzingerode, V., Göbel, U.B., Stackebrandt, E., 1997. Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol. Rev. 21, 213–229. https://doi.org/10.1111/j.1574-6976.1997.tb00351.x.

Yassour, M., Vatanen, T., Siljander, H., Hämäläinen, A.-M., Härkönen, T., Ryhänen, S.J., Franzosa, E.A., Vlamakis, H., Huttenhower, C., Gevers, D., Lander, E.S., Knip, M., Xavier, R.J., 2016. Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Sci. Transl. Med. 8 https://doi.org/10.1126/scitranslmed.aad0917.

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., Heath, A.C., Warner, B., Reeder, J., Kuczynski, J., Caporaso, J.G., Lozupone, C.A., Lauber, C., Clemente, J.C., Knights, D., Knight, R., Gordon, J.I., 2012. Human gut microbiome viewed across age and geography. Nature 486, 222–227. https://doi.org/10.1038/nature11053.