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SYSTEMATIC REVIEW

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Gut microbiome and attention deficit/hyperactivity disorder: a systematic review

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BACKROUND: This systematic review aims to examine the associations between features of gut microbiome and Attention Deficit/Hyperactivity Disorder (ADHD) risk or severity in children, adolescents and young adults.
METHODS: Eligible studies were identified in PubMed and Google Scholar databases until December 31, 2020.
RESULTS: The search identified a total of 1197 items, of which 11 were included in this systematic review. The findings regarding alpha, beta diversity, bacterial phyla, orders and families were inconclusive. At the genus level an increased abundance of *Odoribacter* (two studies) and *Eggerthella* (two studies) was found in ADHD; on the contrary, decreased abundance of *Faecalibacterium* (three studies) was noted, whereas one study suggested its inverse association with ADHD severity and hyperactivity. One study indicated that *Bacteroides species* also correlated with levels of hyperactivity and impulsivity. At the species level, a lower abundance of *Faecalibacterium prausnitzii*, but higher of *Odoribacter splanchnicus* and *Bacteroides uniformis* was reported.
CONCLUSIONS: This systematic review highlights associations between gut microbiome features and ADHD. Potential mechanisms differ by microorganism and include effects on neurotransmitter production, dopamine metabolism, modulation of inflammation and neurodevelopment through the release of cytokines.

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IMPACT:

- The existence of correlations between features of gut microbiome and ADHD manifestation or its severity in children, adolescents and young adults.
- Associations between gut microbiome features and ADHD are highlighted. Potential mechanisms seem to differ by microorganism and include effects on neurotransmitter production, dopamine metabolism, modulation of inflammation and neurodevelopment through the release of cytokines.
- As correlations between gut microbiome features and ADHD seem to exist, additional studies are needed for further investigation.

INTRODUCTION

Attention Deficit/Hyperactivity Disorder is a neurodevelopmental condition with first symptoms appearing early in childhood. Its prevalence is estimated at 5% in children and 2.5% in adults, with a tendency to increase.[1]

According to the latest definition and diagnostic criteria of ADHD in DSM-V by the American Psychiatric Association (DSM-V, 2013), the main characteristics of the disorder involve a persistent pattern of inattention and/or hyperactivity/impulsivity that interfere with everyday actions and development. The three recognized subtypes of ADHD are: combined (ADHD-C), predominantly inattentive (ADHD-I) and predominantly hyperactive/impulsive (ADHD-H).[1] In the International Classification of Diseases 10th edition (ICD-10) by the World Health Organization, ADHD is classified under "Hyperkinetic Disorders".[2]

Clinically ADHD is a condition that appears early in life, before the age of 12 years, consolidates over time and shows great diversity.[3] In adults ADHD demonstrates itself mainly with impulsivity, emotional dysregulation, excessive mind wandering and executive functions deficits, while hyperactivity is more subtle.[4] In many cases, ADHD coexists with comorbidities, such as intellectual disability, communication and learning disorders. The etiology and pathophysiology of ADHD are unclear, but genetics and several environmental risk factors, such as gut microbiome, seem to play a role.[3]

The human gut microbiome is very diverse in comparison to other sites of the body, and there appear to be differences even

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among healthy individuals. Humans are considered to be born sterile and microbial colonization begins immediately at birth. Once established, the microbiome remains relatively stable, but it has been shown that environmental factors, such as nutrition, disease or antibiotics, can cause alterations.[5]

Different indices are used to describe microbial diversity in the human intestinal tract. Alpha-diversity refers to the microbial variation in a sample and it includes number of different species (richness) and equal balance of these species (diversity) in the sample, measured with specific indices. Simpson's and Shannon's indices account for both abundance and evenness of the species present. OTU (count of different species), Observed Species (count of unique OTUs in each sample), Chao1 index and ACE (abundance-based coverage estimators) are richness indices.[6] Beta-diversity is the microbial variation between samples.[7]

When it comes down to the exact composition, the main phyla present in the gut microbiota are Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes with variations in their numbers throughout life. Fusobacteria, Cyanobacteria and Verrucomicrobia are present in smaller numbers.[8] Their representatives in order, family, genus and especially species level can vary significantly among individuals.[5,9] The analysis of the microbiota is performed by a number of methods such as sequencing of the 16 S rRNA-encoding gene and comparison to known databases.[10] Another method is shotgun sequencing, which analyzes the entire microbial community.[8] These two methods are the ones used by the studies included in this review.

The proposed relationship between ADHD and gut microbiome is based on the interplay between the intestinal tract and the central nervous system (CNS), also known as "gut-brain axis", which is now well accepted.[11] This communication interplay involves neural, endocrine and immunal pathways.[12] The autonomic nervous system (ANS), vagus nerve, enteric nervous system (ENS), hypothalamus-pituitary axis (HPA), neurotransmitters, hormones, metabolites and, of course, the gut microflora are implicated in the "gut-brain axis" pathways.[11] Recent studies have linked the gut microbiota with a variety of mental health and neurodevelopmental conditions, such as autism spectrum disorder (ADS).[13–17] Other recent reviews, address the existence of alterations in the gut microbiome of people with ADHD.[18,19] It is important to underline the fact that all studies targeting specifically the relationship between ADHD and the human gut microbiome are very recent and the corresponding reviews quite scarce.

Taking into account the above, the goal of this systematic review is to examine the associations between features of gut microbiome and ADHD risk or severity in children, adolescents and young adults.

MATERIALS AND METHODS

Search algorithm and eligibility criteria

This systematic review was performed following the PRISMA guidelines, in line with an a priori protocol agreed upon and signed by all authors. Eligible studies were identified in PubMed, whereas the first, most relevant 1,000 hits from Google Scholar were also screened. The following search algorithm was implemented: (microbiome OR microbiota OR gut OR microflora OR microbes OR microbe OR micro-organism OR micro-organisms) AND ("attention deficit" OR ADHD). There was no restriction regarding publication language; the end-of-search date was December 31, 2020. References of all eligible articles and relevant review articles were meticulously searched to identify additional studies, in a "snowball" procedure.

Cohort, case-control and cross-sectional studies on humans, comparing gut microbiome features (diversity, phylum, class, order, family, genus and species) in subjects with ADHD versus those without ADHD, or along ADHD severity, were deemed eligible. In addition, interventional studies on probiotic administration to enrich microbiome were also retained but were reported separately. Studies on animals, case reports and case series were excluded. In case of overlapping publications, the largest study and the most recent results were retained. Two authors (DG, KM), working independently to each other, performed the selection of studies and, in case of disagreement, consultation with a third author (TNS) and team consensus was reached.

Data abstraction and evaluation of risk of bias

The following data were abstracted from eligible studies: first author, year of publication, journal name, country in which the study was conducted, study design, study period, sample size, number of subjects with ADHD, number of participants free from ADHD, mean age, age range, selection criteria for the study population, method of ADHD diagnosis, ADHD severity, technique of studying gut microbiome, details about intervention (if applicable), and results pertaining to gut microbiome features (diversity, phylum, class, order, family, genus and species). Data was abstracted into previously piloted forms by two reviewers working blindly to each other (DG, KM); in case of disagreement, consultation with a third author (TNS) and team consensus was reached. The assessment of risk of bias for included studies was performed using the Newcastle-Ottawa scale. Regarding the relevant items pertaining to cohort studies, all available studies have been assessed, whereas attrition rate of 10% or less was deemed adequate.

RESULTS

Selection and description of studies

The search in PubMed and Google Scholar identified a total of 1197 items; after the removal of duplicates, 1085 items were screened in title and abstract Fig. 1. Of these 1043 were excluded as irrelevant from title and abstract. Forty-two were evaluated in full-text; of them, 31 were excluded for reasons presented in Supplementary Table 1. The total number of eligible studies was 11, ten of which were case-control studies performed in children, adolescents and young adults diagnosed with ADHD versus healthy controls. Two of these studies were available online only in pre-print format, prior to completion of peer review.[20,21] One interventional study investigated the development of ADHD and the differences of gut microbiome after the administration of probiotics to a cohort of children, versus a placebo group.[22] Description of eligible studies is provided in Table 1.

Alpha-diversity and beta-diversity: ADHD cases versus controls

Results of individual studies are presented in detail in Table 2. Alpha-diversity was examined in eight studies; in five studies[21,23–26] no significant difference was found in ADHD cases versus controls. However, Li et al. (2020)[21] revealed lower gene numbers in the ADHD group compared to HCs, but no difference in alpha diversity. Prehn-Kristensen et al.[27] found one index (Shannon) lower in ADHD cases, but the other indexes (Chao1, observed species) showed no difference. In the study by Wang et al. (2019)[28] two indexes (Shannon, Chao1) were higher in ADHD subjects, one index (Simpson) was lower and one (ACE) was similar in both groups.

Fan et al. (2019)[20] showed that three indexes (ACE, Shannon, Chao1) were lower in the ADHD-I group while Simpson Index was higher in the ADHD-I and the ADHD-C groups, compared to healthy controls.

Beta-diversity was examined in eight studies; of them, four[23,24,26,28] showed no significant difference whereas the remaining four[20,22,25,27] showed compositional differences between the two groups. Szopinska-Tokov (2020)[25] specified that the ADHD group had a smaller variation in the gut microbiota

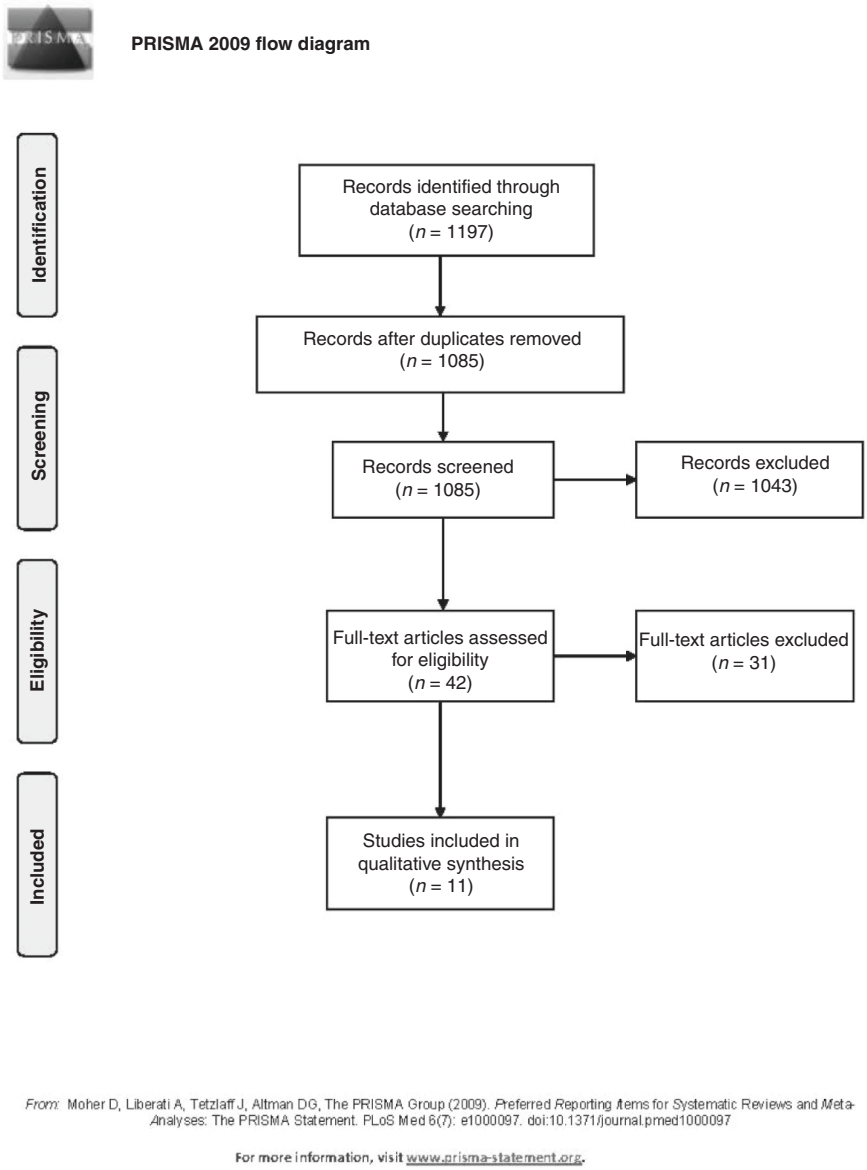

Fig. 1 Prisma Flow Chart. The steps regarding the selection of eligible studies are presented in the following chart, according to Prisma guidelines.

composition, which means a higher taxonomic similarity (within the group) compared to healthy controls; however, the remaining three studies did not provide the relevant details.[20,21,27]

Microbial phylum, class, order and family differences in ADHD versus controls

The microbiota composition at the phylum level was evaluated in eight studies. Of them, four[24,25,27,29] found no difference. Aarts et al. (2017)[23] showed an increase in *Actinobacteria* and a decrease in *Firmicutes* in ADHD subjects versus controls. Li et al. (2020)[21] showed that healthy controls had higher numbers of *Fusobacteria*, while Wang et al. (2019)[28] revealed higher numbers of *Fusobacteria* in the ADHD group. When it comes to ADHD subgroups, only Fan et al. (2019)[21] suggested decreased numbers of *Verrucomicrobia* in ADHD-I.

None of the eligible studies gave information about the difference in abundance of microbial classes between ADHD and healthy controls.

Information about the microflora composition at the order level was investigated in four studies. Aarts et al. (2017)[23] found *Clostridiales*, within the phylum *Firmicutes*, to be decreased in ADHD and *Bifidobacteriales* increased. On the other hand, Cheng et al (2019)[28] found *Clostridiales* increased in ADHD. Akram et al. (2017)[29] reported differences in *Burkholderiales*, *Alcaligenaceae* and *Erysipelotrichaceae* without any further details. Li et al. (2020)[21] mentioned lower numbers of *Fusobacteriales*, *Flavobacteriales*, *Rhodocyclales* and *Sphigomonadales* in ADHD and especially in ADHD-I lower *Rhizobiales*, versus controls.

The microbial families represented in the gut microbiome were reported in five case-control studies. Aarts et al. (2017)[23] found that *Rikenellaceae*, *Porphyromonadaceae* and *Bifidobacteriaceae* were more abundant in ADHD, while *Ruminococcaceae* and *Lachnospiraceae* were increased in healthy controls. Jiang et al. (2018)[24] found a lower percentage of *Alcaligenaceae* and higher of *Peptostreptococcaceae*, *Moraxellaceae*, *Xanthomonadaceae*, and *Peptococcaceae* in the ADHD group. Prehn-Kristensen et al. (2018)[27] found elevated levels of *Prevotellaceae*, *Catabacteriaceae*, and *Porphyromonadaceae* in healthy controls, but *Neisseriaceae* and *Bacteroidaceae* in ADHD children. In the study by Li et al. (2020),[21] *Fusobacteriaceae*, *Flavobacteriaceae*, *Rhodocyclaceae*,

Table 1. Characteristics of included studies.

First author (year)	Region, country	Language	Study period	Study design	Sample size	Type of intervention	No exposed	No ADHD	No controls	Mean age years	Age range years	Study population	Method of ADHD diagnosis	Method of studying gut microbiome	Time of evaluation
Aarts[23]	The Netherlands	English	Not mentioned	Case- control	96	n/a	n/a	19	77	19.5 for ADHD, 27.1 for HC	Not mentioned	Not mentioned	DSM-IV symptoms using the Schedule for Affective Disorders and Schizophrenia for School-Age Children	16 S rRNA marker gene sequencing (16 S) to identify bacterial taxa and their pre- dicted gene functions	n/a
Akram[29]	USA	English	Not mentioned	Case- control	34 (19 female, 15 males)	n/a	n/a	14	20	Not mentioned	18–28	Not mentioned	Adult ADHD Self-Reported (ASRS) Questionnaire	Bacterial 16 S ribosomal RNA gene targeted sequencing was performed	n/a
Cheng (2019)	China	English	Not mentioned	Case- control	53,293	n/a	n/a	19 099	34 194	Not mentioned	Not mentioned	European whites downloaded from the Psychiatric GWAS Consortium website (https://www.med.unc.edu/pgc/results-and-downloads)	DSM	The gut microbiota-associated host genes were collected from recently published GWAS of gut microbiota (GWASGM)	n/a
Fan[20]	China	English	6/2017-5/2018	Case- control	73	n/a	n/a	49 (7 females)	24 (3 females)	Not mentioned	6–12	Chinese	ADHD children met the DSM-IV diagnosis and scored above clinical threshold for ADHD symptoms on both the Strength and Difficulty Questionnaire (SDQ)	16 S rRNA gene 44 sequencing,	n/a
Jiang[24]	China	English	5/2015-12/2016	Case- control	83	n/a	n/a	51 (13 females)	32 (10 females)	8.47 for ADHD, 8.5 for HC	6–10	Chinese	Kiddie-SADS-Present and Lifetime Version (Kiddie-SADS -PL) scale, which is a semi-structured diagnostic interview conducted according to the DSM-IV. The parents of all participating children completed the Conners Parent Rating Scales (CPRS) to assess the severity of ADHD symptoms	Fecal microbial DNA was extracted from 200 mg of feces using the QIAamp DNA Stool Mini Kit (Qiagen; Hilden, Germany), in conjunction with additional glass-bead beating steps on a Mini-beadbeater (FastPrep; Thermo Electron Corp.; Boston, MA, USA).	n/a
Li[21]	China	English	20/3/2018-27/2/2020	Case- control	207	n/a	n/a	98	109	9 for ADHD, 8.9 for HC	6–15	Chinese	DSM IV	Total bacterial DNA from each fecal sample was extracted with the NucleoSpin (R) Soil kit.The final DNA libraries were assessed and quantified by a Real-Time PCR system. The profile of microbial composition for each sample was calculated using MetaPhlAn2(v2.0) to estimate the relative abundance of bacterial taxa.	n/a

Table 1. continued

First author (year)	Region, country	Language	Study period	Study design	Sample size	Type of intervention	No exposed	No ADHD	No controls	Mean age years	Age range years	Study population	Method of ADHD diagnosis	Method of studying gut microbiome	Time of evaluation
Prehn-Kristensen[27]	Kiel, Germany	English	Not mentioned	Case- control	31	n/a	n/a	14 male	17 male	11.9 for ADHD, 13.1 for HC	Not mentioned	Caucasians	Children andir parents were interviewed using a German translation of the Revised Schedule for Affective Disorders and Schizophrenia for School-Age Children: Present and Lifetime Version (K-SADS-PL), the Child Behavior Checklist (CBCL) and the German ADHD rating scale (Fremdbeurteilungsbogen für hyperkinetische Störungen, FBB-HKS), were completed by parents to assess any psychiatric symptoms in their children. According to the DSM-IV-TR, all patients met the criteria for ADHD	Next generation sequencing of 16 S rDNA and analyzed for diversity and biomarkers, Total DNA from fecal samples was extracted using FastDNATM SPIN KIT FOR SOIL (Qbiogene, Carlsbad, CA, USA)	n/a
Szopinska-Tokov[25]	The Netherlands	English	Not mentioned	Case- control	107	n/a	n/a	41 (+15) subthreshold ADHD	47	20.2 for ADHD, 20.5 for HC	13–29	Dutch (Caucasian)	A semi-structured diagnostic interview of DSM-IV criteria was conducted with both the participant and his/her parents using the Kiddie-Schedule for Affective Disorders and Schizophrenia (K-SADS) according to DSM-IV criteria. Clinical diagnosis was confirmed using a diagnostic algorithm which combined the diagnostic interview (K-SADS) with the Conners rating scales. Continuous measures of inattention severity (IA) and hyperactivity/impulsivity severity (HI) were derived from the Conners Adult ADHD Rating Scales (CAARS; ≥ 16 years) and Conners Teacher Rating Scale (CTRS; < 16 years).	The human fecal samples were collected at home by the participants and stored at 4 °C. DNA purification was performed with a customized kit (AS1220; Promega, Leiden, The Netherlands). The purified bacterial DNA was measured with a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA), and aliquots of 20 ng/μL were prepared for the 2-step Polymerase Chain Reaction (PCR) reactions (including negative controls)	n/a
Wan[26]	Beijing, China	English	1/2019-6/2019	Case- control	34	n/a	n/a	17 (14 males, 3 females)	17 (13 males, 4 females)	8	6–12	Not mentioned	Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS, Present and Lifetime Version scales) and the diagnostic criteria in the (DSM-5) based on the opinion of an experienced child psychiatrist	Fecal samples of cases and controls were analyzed by shotgun metagenomics sequencing.	n/a
Wang (2019)	Taiwan	English	Not mentioned	Case-control	60	n/a	n/a	30 (23 males, 7 females)	30 (18 males, 12 females)	8.4 for ADHD, 9.3 for HC	6–16	Not mentioned	DSM-IV-TR and K-SADS-E by a senior psychiatrist	Fecal samples were investigated using 16 S rRNA V3V4 amplicon sequencing	n/a

Table 1. continued

First author (year)	Region, country	Language	Study period	Study design	Sample size	Type of intervention	No exposed	No ADHD	No controls	Mean age years	Age range years	Study population	Method of ADHD diagnosis	Method of studying gut microbiome	Time of evaluation
Pärtty[22]	Finland	English	2/1997-1/1998	Randomized double-blind, placebo-controlled prospective follow-up	75	The mothers of these children received 1 × 10 (10) colony -forming units of Lactobacillus rhamnosus GG or placebo (microcrystalline cellulose) daily for 4 wk before expected delivery. After delivery, the capsule contents were given either to the children, or continuously to the mothers, if breast- feeding, for 6 months.	40	n/a	35	infants	Not mentioned	Not mentioned	The diagnostic criteria of ICD-10 were used.	A fecal sample was taken at 3 wk, 3, 6, 12, 18, 24 mo, and 13 y. They were pretreated and DNA was extracted using an automated KingFisher DNA extraction system. Quantitative PCRs were conducted. Bacterial cells were harvested and fixed and FISH carried out with fluorophore (indocarbocyanine Cy3)-labeled oligonucleotide probes. Total cell numbers were determined by nucleic acid stain 4′, 6-diamidino-2-phenylindole (DAPI). Cells were counted visually using an Olympus SZX9 epifluorescence microscope.	3 months, 6 months, 12 months, 18 months, 24 months, 13 years

Sphigomonadaceae and *Bacillales* noname were less abundant in the ADHD patients than in healthy controls, while *Prevotellaceae* was more abundant in the ADHD patients. In regard to ADHD subgroups, the study concluded that *Oscillospiraceae* was more frequently encountered in healthy controls and ADHD-I compared to ADHD-C, whereas *Listeriaceae*, *Prevotellaceae* and *Veillonellaceae* were more frequently encountered in ADHD-C compared with ADHD-I or HC. According to Fan et al. (2019)[20] *Ruminococcaceae* were decreased in ADHD-I and ADHD-H; *Lachnospiraceae*, *Verrucomicrobiaceae* and *Rikenellaceae* were decreased in the ADHD-I group and *Prevotellaceae* were increased in ADHD-C and ADHD-H, versus controls.

Microbial genus and species in ADHD cases versus controls

Regarding the genus level, all eligible case-control studies reported the relevant results. According to the findings by Aarts et al. (2017),[23] *Bifidobacterium*, *Eggerthella*, *Alistipes*, *Parabacteroides* and *Odoribacter* were increased in ADHD, whereas *Subdoligranulum*, *Ruminococcus* and *Coprococcus* were more abundantly encountered in healthy controls. Akram et al. (2017)[29] reported differences in *Phascolarctobacterium*, *Paraprevotella*, *Veillonella* and *Odoribacter*, without further specifications. According to Cheng (2019),[30] *Desulfovibrio* was more abundant in ADHD. According to Prehn-Kristensen et al. (2018),[27] *Prevotella* and *Parabacteroides* were detected as markers for the control group and *Neisseria* for the ADHD group. In the study by Szopinska-Tokov et al. (2020),[25] *Ruminoclostridium 9*, *Ruminococcus 2*, *Clostridiales g*, *Ruminococcaceae* NK4A214 group, *Ruminococcaceae* UCG 003, *Ruminococcaceae* UCG 004, *Ruminococcaceae* UCG 005, *Ruminococcaceae* g uncultured, Family XIII AD3011 group were more frequent in ADHD and *Haemophilus* in HC. According to the findings by Wan et al. (2020),[26] *Faecalibacterium* and *Veillonella* were significantly reduced in the ADHD group, while *Odoribacter* and *Enterococcus* were significantly more abundant. Wang et al. (2019)[28] concluded that *Fusobacterium* was elevated in the ADHD group, while the relative abundance of *Lactobacillus* was enriched in healthy controls. Finally, Jiang et al. (2018)[24] reported decreased numbers of *Faecalibacterium*, *Lachnoclostridium*, *Sutterella* and *Dialister* in the ADHD group.

Fan et al. (2019)[20] found greater abundance of *Megamonas*, *Coprococcus 2* and *Paraprevotella* in the ADHD-C, relatively to healthy controls. The aforementioned study also mentioned lower percentage of *Faecalibacterium* and higher of *Marvinbryantia*, *Intestinimonas*, *Prevotella 9* and *Eggerthella* in ADHD-H subjects compared to healthy controls, but reduced *Akkermansia*, *Ruminococcaceae* UCG002, *Lachnospiraceae* NK4A136 group, *Eubacterium coprostanoligenes* group, *Christensenellaceae* R-7 group, *Ruminococcaceae* UCG014, *Ruminococcaceae* UCG005, Unclassified f *Lachnospiraceae*, *Anaerotruncus*, *Coprococcus 1*, *Ruminoclostridium 5*, *Alistipes*, norank f *Bacteroidales* S24-7 group in ADHD-I. Li et al. (2020)[21] found *Prevotella* and *Scardovia* more frequent in ADHD, whereas *Subdoligranulum*, *Phascolarctobacterium*, *Adlercreutzia*, *Fusobacterium*, *Gemella*, *Methyloversatilis* and *Brevundimonas* were more frequent in healthy controls. Regarding ADHD subgroups, the study found that *Prevotella* and *Listeria* were more frequent in ADHD-C, while *Bifidobacterium*, *Subdoligranulum*, *Bilophila*, *Oscillibacter* and *Acidaminococcus* were more frequent in ADHD-I.

Four studies presented information about the species composition. Aarts et al. (2017)[23] concluded that *Coprococcus eutactus* was increased in HC, *Bacteroides* (*vulgatus*, *ovatus*, *uniformis*), and *Bifidobacterium* (*longum*, *adolescentis*, *pseudocatenulatum*) were increased in ADHD. In the study by Wan et al. (2020)[26] *Faecalibacterium prausnitzii*, *Lachnospiraceae* bacterium, and *Ruminococcus gnavus* numbers were significantly decreased in the ADHD group, while *Bacteroides caccae*, *Odoribacter splanchnicus*, *Paraprevotella xylaniphila*, and *Veillonella parvula* were significantly increased. Wang et al. (2019)[28] found that *Bacteroides coprocola* in the ADHD group was significantly less frequent than in the control

Table 2. Results of included studies.

First author (year)	alpha- diversity	beta- diversity	Phylum	Order	Family	Genus	Species	Effect on severity of ADHD	Outcome
Aarts[23]	No significant differences [Chao 1 index: mean ADHD = 604.5, mean HC = 579.7, p = non significant. Shannon index: mean ADHD = 5.3, mean HC = 5.2, p = non significant]	No significant differences	In both groups the main phyla were: Firmicutes (77.92%), Actinobacteria (15.68%) and Bacteroidetes (6.05%). Increase of Actinobacteria (HC:14.08% to ADHD:22.14%; p = 0.002) at the expense of Firmicutes (HC:79.80% and ADHD:70.29%; p = 0.001), as Bacteroidetes did not differ significantly (HC:5.74% to ADHD:7.29%; p = 0.166,) Proteobacteria also did not differ significantly.	Clostridiales, within the phylum Firmicutes, was found to be decreased in ADHD cases (HC:77.37% to ADHD: 69.02%; p = 0.003). Bifidobacteriales was increased in ADHD ($p < 0.01$)	Rikenellaceae, Porphyromonadaceae and Bifidobacteriaceae were increased in ADHD ($p < 0.01$). Ruminococcaceae, Lachnospiraceae were increased in HC ($p < 0.05$)	Bifidobacterium was significantly increased in ADHD (HC:12.66% to ADHD:20.47%; p = 0.002), Eggerthella was slightly increased in ADHD ($p <$ 0.001), as were Alistipes, Parabacteroides, Odoribacter ($p < 0.05$). Subdoligranulum, Ruminococcus, Coprococcuswere increased in HC ($p < 0.05$)	Coprococcus eutactus was increased in HC ($p < 0.01$), Bacteroides (vulgatus, ovatus, uniformis), Bifidobacterium (longum, adolescentis, pseudocatenulatum) were increased in ADHD ($p < 0.05$)	Not mentioned	n/a
Akram[29]	Not mentioned	Not mentioned	Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria were the main phyla in both groups with no significant difference.	Burkholderiales, Alcaligenaceae, Erysipelotrichaceae were different in the two groups	Not mentioned	Phascolarctobacterium, Paraprevotella, Veillonella, Odoribacter were different in the two groups	Not mentioned	Not mentioned	n/a
Cheng (2019)	Not mentioned	Not mentioned	Not mentioned	Clostridiales higher in ADHD (p = 0.034)	Not mentioned	Desulfovibrio higher in ADHD (p = 0.031)	Not mentioned	Not mentioned	n/a
Fan[20]	Compared to HC (mean ± SD: Chao1 = 220.48 ± 46.32, ACE = 215.91 ± 43.47, Shannon = 3.39 ± 0.36, Simpson = 0.07 ± 0.03) ACE (176,34 ± 49.72), Chao1 (176.49 ± 49.87), Shannon (2.83 ± 0.38) were lower in ADHD-I group and Simpson (0.14 ± 0.07) was higher in ADHD-I group and also Simpson was higher (0.19 ± 0.05) in ADHD-C group. ($p < 0.05$).	Compositional differences between the two groups	In both groups the main phyla were Firmicutes and Bacteroidetes, followed by Proteobacteria, Actinobacteria and Verrucomicrobia. only children in ADHD-I group have a significant lower Verrucomicrobia (ascribed to the reduced Akkermansia genus)	Not mentioned	Ruminococcaceae significantly decreased in ADHD-I and ADHD-H, Lachnospiraceae, Verrucomicrobiaceae and Rikenellaceae were decreased in the ADHD-I group. Increased abundance of Prevotellaceae in ADHD-C and ADHD-H	Greater abundance of Megamonas, Coprococcus 2 and Paraprevotella in the ADHD-C relative to HC. Lower percentage of Faecalibacterium, and higher of Marvinbryantia, Intestinimonas, Prevotella 9 and Eggerthella in the ADHD-H compared to HC. Reduced Akkermansia, Ruminococcaceae UCG002, Lachnospiraceae NK4A136 group, Eubacterium coprostanoligenes group, Christensenellaceae R-7 group, Ruminococcaceae UCG014, Ruminococcaceae UCG005, Unclassified f Lachnospiraceae, Anaerotruncus, Coprococcus 1, Ruminoclostridium 5, Alistipes, norank f Bacteroidales S24-7 group in ADHD-I ($p < 0.05$)	Not mentioned	Not mentioned	n/a
Jiang[24]	No significant differences. ACE: mean ADHD = 247.01(54.646), mean HC = 229.08 (52.249), p = 0.143. Chao1 Index: mean	No significant differences	No significant differences (the main Phyla in both groups: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria) $p < 0.05$	Not mentioned	Lower percentage of Alcaligenaceae but a greater abundance of Peptostreptococcaceae, Moraxellaceae, Xanthomonadaceae, and Peptococcaceae in the ADHD group ($p < 0.05$)	Faecalibacterium, Lachnoclostridium, Sutterella and Dialister, were decreased in the ADHD group ($p < 0.05$)	Not mentioned	Faecalibacterium negatively associated with ADHD severity and hyperactivity	n/a

Table 2. continued

First author (year)	alpha- diversity	beta- diversity	Phylum	Order	Family	Genus	Species	Effect on severity of ADHD	Outcome
	ADHD = 246.12 (58.394), mean HC = 229.97 (54.492), p = 0.212. Shannon index: mean ADHD = 3.361 (0.437), mean HC = 3.337(0.398), p = 0.8. Simpson Index: mean ADHD = 0.082 (0.041), mean HC = 0.081(0.04), p = 0.974								
Li[21]	We found significantly lower gene numbers in ADHD patients than in HCs (p = 0.042), though the gut microbiota richness was similar between the two groups (Shannon index, p = 0.076). Lower dissimilarity between I-ADHD and HC (p = 0.11) than between C-ADHD and HC (p = 0.017). However, alpha diversity analysis revealed no significant difference between any two groups among C-ADHD, I-ADHD and HC.	significant dissimilarity between C-ADHD and HC (p = 0.020)	Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria were the most abundant taxa in both groups. The HCs displayed a higher abundance of Fusobacteria than the ADHD patients (mean ± SD: ADHD = 0.03 ± 0.09, HC = 0.22 ± 1.19, p = 0.01)	Fusobacteriales, Flavobacteriales, Rhodocyclales and Sphigomonadales were lower in the ADHD patients than in the HCs. *Subgroups:* Rhizobiales was higher in HC and I-ADHD compared to C-ADHD (p < 0.05)	Fusobacteriaceae, Flavobacteriaceae, Rhodocyclaceae, Sphigomonadaceae and Bacillales noname were lower in the ADHD patients than in the HCs, Prevotellaceae was higher in the ADHD patients. *Subgroups:* Oscillospiraceae was higher in HC and I-ADHD compared to C-ADHD (p < 0.05). Listeriaceae, Prevotellaceae and Veillonellaceae were higher in C-ADHD compared with I-ADHD or HC (p < 0.05).	Prevotella (mean ± SD: ADHD = 10.8422 ± 23.6450, HC = 5.7004 ± 16.6239, p = 0.0001) and Scardovia (mean ± SD: ADHD = 0.0011 ± 0.0025, HC = 0.0007 ± 0.0018, p = 0.0423) were higher in ADHD while Subdoligranulum (mean ± SD: ADHD = 1.0550 ± 1.4859, HC = 1.5567 ± 2.0861, p = 0.0158), Phascolarctobacterium (mean ± SD: ADHD = 0.0515 ± 0.3612, HC = 0.0478 ± 0.2903, p = 0.0173), Adlercreutzia (mean ± SD: ADHD = 0.0392 ± 0.0812, HC = 0.0554 ± 0.0862, p = 0.0177), Fusobacterium (mean ± SD: ADHD = 0.0257 ± 0.0937, HC = 0.2223 ± 1.1925, p = 0.0065), Gemella (mean ± SD: ADHD = 0.0028 ± 0.0062, HC = 0.0031 ± 0.0045, p = 0.0114), Methyloversatilis (mean ± SD: ADHD = 0.0006 ± 0.0013, HC = 0.0008 ± 0.0014, p = 0.0216) and Brevundimonas (mean ± SD: ADHD = 0.0001 ± 0.0005, HC = 0.0003 ± 0.0007, p = 0.0432) were higher in HC. *Subgroups:* Prevotella (mean ± SD: I-ADHD = 7.7403 ± 21.4784, C-ADHD = 14.3709 ± 26.1057, HC = 5.7004 ± 16.6239, p = 0.0004) and Listeria (mean ± SD: I-ADHD = 0.0000 ± 0.0001, C-ADHD = 0.0002 ± 0.0006,HC = 0.0000 ± 0.0001, p = 0.0027) were higher in C-ADHD while Bifidobacterium (mean ± SD: I-ADHD = 3.5763 ± 3.3021, C-ADHD = 2.3468 ± 2.9024, HC = 2.6185 ± 2.5194, p = 0.0357), Subdoligranulum (mean ± SD: I-ADHD = 1.4957 ± 1.7370, C-ADHD =	Bacteroides (ovatus, fragilis, thetaiotaomicron, intestinalis, cellulosilyticus, salyersiae, fluxus, nordii) are higher in HC, Bifidobacterium (breve, bifidum) and Prevotella (amnii, buccae, copri) are higher in ADHD (p < 0.05). *Subgroups:* Bacteroides (cellulosilyticus, fluxus, nordii, ovatus), Lachnospiraceae bacterium, Bilophila wadsworthia, Oscillibacter unclassified and Subdoligranulum unclassified were higher in HC and I-ADHD compared to C-ADHD (p < 0.05). Listeria marthii was higher in C-ADHD compared to I-ADHD or HC (p < 0.05).	The abundant species in C-ADHD compared with HC, Prevotella buccae, Bifidobacterium breve, and Bifidobacterium bifidum were enriched and positively associated with the scores of both total CPRS and DSM. Increased relative abundances of Bacteroides nordii, Bacteroides cellulosilyticus and Bacteroides intestinalis were associated with fewer symptoms in both hyperactivity/ Impulsivity (DSM HD scores) and inattention (DSM AD scores). While Bacteroides thetaiotaomicron and Bacteroides ovatus were negatively associated only with DSM AD scores.	n/a

Table 2. continued

First author (year)	alpha- diversity	beta- diversity	Phylum	Order	Family	Genus	Species	Effect on severity of ADHD	Outcome
						0.8076 ± 1.3133, HC = 1.5567 ± 2.0861, p = 0.0014), Bilophila (mean ± SD: I-ADHD = 0.4680 ± 0.5646,C-ADHD = 0.2805 ± 0.4379, HC = 0.3801 ± 0.3711, p = 0.035) Oscillibacter (mean ± SD: I-ADHD = 0.6648 ± 0.9545, C-ADHD = 0.2475 ± 0.3046, HC = 0.4252 ± 0.4613, p = 0.0007) and Acidaminococcus (mean ± SD: I-ADHD = 0.0384 ± 0.1357, C-ADHD = 0.0048 ± 0.0281, HC = 0.0071 ± 0.0276, p = 0.0059) were higher in I-ADHD compared with C-ADHD.			
Prehn-Kristensen[27]	Shannon index was significantly decreased in ADHD compared to controls (p = 0.036), while observed species (p = 0.25) and Chao 1 index (p = 0.17) showed no difference.	Dissimilarities in the microbial composition were found (p = 0.033).	No significant differences (the main Phyla in both groups: Firmicutes, Bacteroidetes, Proteobacteria, Tenericutes, Actinobacteria)	Not mentioned	Elevated levels of Prevotellaceae, Catabacteriaceae, and Porphyromonadaceae for healthy controls and Neisseriaceae andBacteroidaceae for the ADHD children.	**Prevotella** and **Parabacteroides** were detected as markers for the control group and **Neisseria** for the ADHD group.	Not mentioned	Levels of hyperactivity were significantly correlated with a change in alpha diversity (p = 0.03), **Bacteroides** species levels correlated with levels of hyperactivity and impulsivity	n/a
Szopinska-Tokov[25]	No significant difference. [observed OTUs (p = 0.08), Shannon index (p = 0.16) and Phylogenetic diversity (p = 0.15)]	The ADHD group had a smaller variation in the gut microbiota composition, which means a higher taxonomic similarity(within the group) compared to HC (p = 0.004)	No significant differences (Firmicutes, Bacteroides, Actinobacteria, Proteobacteria and Verrucomicrobia were the main phyla in both groups)	Not mentioned	Not mentioned	**Ruminoclostridium 9, Ruminococcus2, Clostridiales g, Ruminococcaceae NK4A214 group, Ruminococcaceae UCG 003,Ruminococcaceae UCG 004, Ruminococcaceae UCG 005, Ruminococcaceae g uncultured, Family XIII AD3011 group** are increased in ADHD and **Haemophilus** is increased in HC	Not mentioned	The variation in b-diversity was significantly explained by disorder status (p = 0.033) and inattention score (p = 0.014), while hyperactivity/ impulsivity score was at trend level (p = 0.059). **Ruminococcus 2** [B(SE):1.525 (0.454), 95%CI:0.675–2.362, p = 0.001] and **Ruminococcaceae UCG_004** [B(SE): 43.920 (13.348), 95% CI:19.372–69.095, p = 0.001] was associated with inattention scores. No association of tested genera and hyperactivity/ impulsivity.	n/a
Wan[26]	No significant differences. Shannon index (ADHD = 9.67 ± 0.42, HC = 9.52 ± 0.25), Chao1 index (ADHD = 61.5 ± 11.6, HC = 57.5 ± 9.8) and Simpson index (ADHD = 0.89 ± 0.07, HC = 0.88 ± 0.06)	No discrimination between groups	Not mentioned	Not mentioned	Odoribacteraceae and Enterococcaceae were significantly increased in the ADHD group, while Ruminococcaceae was significantly decreased	**Faecalibacterium** and **Veillonella** were significantly reduced in the ADHD group, while **Odoribacter** was significantly higher (p < 0.05). **Enterococcus** was significantly increased in the ADHD group	**Faecalibacterium prausnitzii, Lachnospiraceae bacterium**, and **Ruminococcus gnavus** were significantly decreased in the ADHD group, while **Bacteroides caccae, Odoribacter splanchnicus, Paraprevotella xylaniphila**, and	Not mentioned	n/a

Table 2. continued

First author (year)	alpha- diversity	beta- diversity	Phylum	Order	Family	Genus	Species	Effect on severity of ADHD	Outcome
							Veillonella parvula were significantly increased ($p < 0.05$)		
Wang (2019)	Chao 1 index was increased in ADHD (mean ADHD = 2789 ± 212.0, mean HC = 2144 ± 168.1, p = 0.0351), Shannon index was also increased in ADHD (mean ADHD = 2.92 ± 0.08, mean HC = 2.65 ± 0.08, P = 0.0378), Simpson index was lower in ADHD (mean ADHD = 0.13 ± 0.01, mean HC = 0.17 ± 0.01, p = 0.0339). ACE was similar in both groups (mean ADHD = 7587 ± 667.3, mean HC = 5933 ± 514.3, p = 0.0905).	Similar	Fusobacteria was higher in the ADHD group[median = 0.28% (0.02–3.28) in ADHD and 0.02% (0.00–0.45) in HC and p = 0.041], The healthy controls demonstrated a dominance of Bacteroidetes [median = 72.04 (63.51–76.63) in ADHD and median = 73.68% (67.82–80.64) in HC, p = 0.220], followed by Firmicutes [median = 10.61 (8.15–12.97) in ADHD, median = 11.71(8.06–16.20) in HC, p = 0.9471], Proteobacteria [median = 10,32 (4.83–13.21) in ADHD and median = 8.21 (5.50–11.61) in HC, p = 0.311], Fusobacteria (0.02%), and Actinobacteria [median = 0.36 (0.17–2.35) in ADHD and median = 0.33 (0.01–1.15) in HC, p = 0.225]. These phyla represent 99% of all bacteria in both groups.	Not mentioned	Not mentioned	**Fusobacterium** was elevated in the ADHD group [median = 0.28 (0.02–3.28) in ADHD and median = 0.02 (0.00–0.45) in HC, p = 0.041], while the relative abundance of **Lactobacillus** was enriched in healthy controls.	**Bacteroides coprocola** in the ADHD group was significantly lower than in the control group (p = 0.028), while the relative abundance of **Bacteroides uniformis** (p = 0.021), **Bacteroides ovatus** (p = 0.023), and **Sutterella stercoricanis** (p = 0.001) in the ADHD group were significantly higher than in the control group.	the increased proportion of **B. uniformis, B.ovatus and S. stercoricans** and decreased proportion of **B. coprocola** may be associated with susceptibility to ADHD	n/a
Pärtty[22]	Not mentioned	Not mentioned	At the age of 18 mo: the mean (SD) numbers of Bacteroides and Lactobacillus-Enterococcus group bacteria were lower among children with ADHD/AS than healthy children (7.28) (0.85) log cells/g vs. 8.13 (0.51) log cells/g, P = 0.008; 7.71 (0.78) log cells/g vs. 8.40 (0.40) log cells/g, P = 0.01, respectively.	Not mentioned	Not mentioned	*At the age of 6 mo (when probiotic intervention was completed):* the mean (SD) number of cells belonging to the genus **Bifidobacterium** was significantly lower among children with neuropsychiatric disorders than in those without (8.26 (1.24) log cells/g vs. 9.12 (0.64) log cells/g, respectively, P = 0.03).	*At the age of 3 mo:* significantly lower median numbers of **Bifidobacterium longum** among the children with neuropsychiatric disorders than in healthy children (4.35 (3.99–10.40) log/g and 10.18 (8.88–10.88) log/g, respectively, P = 0.045). *At the age of 24 mo:* the mean (SD) numbers of cells belonging to the **Clostridium histolyticum** group were lower among children with ADHD or AS than in healthy children, (7.46 (0.44) log cells/g vs. 8.16 (0.55) log cells/g; P = 0.04).	n/a	*At the age of 13y:* 3 children were diagnosed with ADHD (4%) and 2 children with both ADHD and ASD (2.7%). All these children were in the placebo group (p = 0.008). *At the age of 13:* there were no statistically significant differences in gut microbiota composition between children with or without neuropsychiatric disorders

Table 3. Table showing the most important findings on gut microbiome composition, between ADHD cases and controls.

studies	Aarts[23]	Akram[29]	Cheng (2019)	Fan[20]	Jiang[24]	Li[21]	Prehn-Kristensen[27]	Szopinska-Tokov[25]	Wan[26]	Wang (2019)	Partty[22]
a-diversity											
Shannon index				lower in ADHD-I			lower				
b-diversity				differences			differences	lower variation			differences
Family											
Prevotellaceae				higher in ADHD-C		higher in ADHD-C					
genera											
Odoribacter	higher	difference							higher		
Eggerthella	higher			higher							
Faecalibacterium				lower	lower				lower		
species											
B.uniformis	higher						higher				

Only results, for which at least two separate studies have agreed upon, are displayed. Empty boxes correspond to lack of information or no agreement between studies.
ADHD - I inattentive subtype of ADHD, *ADHD - C* combined subtype of ADHD.

group, while the relative abundance of *Bacteroides uniformis*, *Bacteroides ovatus*, and *Sutterella stercoricanis* in the ADHD group were significantly more frequent than in the control group.

According to Li et al. (2020),[21] Bacteroides (ovatus, fragilis, thetaiotaomicron, intestinalis, cellulosilyticus, salyersiae, fluxus, nordii) were more abundant in healthy controls, whereas Bifidobacterium (breve, bifidum) and Prevotella (amnii, buccae, copri) were more abundant in ADHD. Especially regarding ADHD subgroups, Bacteroides (cellulosilyticus, fluxus, nordii, ovatus), Lachnospiraceae bacterium, Bilophila wadsworthia, Oscillibacter unclassified and Subdoligranulum unclassified were retrieved in higher numbers in healthy controls and I-ADHD compared to C-ADHD, whereas Listeria marthii was more abundant in C-ADHD compared to I-ADHD or healthy controls.

Gut microbiome and ADHD severity

Associations with the severity of ADHD were reported in four studies. Jiang et al. (2018)[24] concluded that *Faecalibacterium* was negatively associated with ADHD severity and hyperactivity. According to Li et al. (2020),[21] the abundant species *Prevotella buccae*, *Bifidobacterium breve* and *Bifidobacterium bifidum* in ADHD-C compared with healthy controls, were enriched and positively associated with the results regarding ADHD of both total Conners Parent Rating Scales (CPRS) and DSM. Increased relative abundances of *Bacteroides nordii*, *Bacteroides cellulosilyticus* and *Bacteroides intestinalis* were associated with fewer symptoms in both hyperactivity/impulsivity and inattention, while *Bacteroides thetaiotaomicron* and *Bacteroides ovatus* were negatively associated only with inattention scores. In the study by Prehn-Kristensen et al. (2018),[27] levels of hyperactivity significantly correlated with a change in alpha-diversity, whereas *Bacteroides* species levels correlated with levels of hyperactivity and impulsivity. According to Szopinska-Tokov et al. (2020),[25] the variation in beta-diversity was explained by disorder status and inattention level. Also *Ruminococcus 2* and *Ruminococcaceae* UCG 004 were associated with higher inattention levels.

Randomized trial results

In regard to the randomized, double-blind,placebo-controlled prospective follow-up study by Pärtty et al. (2015),[22] at the age of 13 years, three children out of 75 total were diagnosed with ADHD (4%) and two children with both ADHD and ASD (2.7%); all these children were in the placebo group. At the age of 3 months, significantly lower median numbers of *Bifidobacterium longum* were noted among the children with neuropsychiatric disorders versus healthy children. At the age of 6 months, when the probiotic intervention was completed, the genus *Bifidobacterium* was significantly less frequent among children with neuropsychiatric disorders than in those without. At the age of 18 months, *Bacteroides* and *Lactobacillus-Enterococcus* group bacteria were less frequent among children with ADHD versus healthy children. At the age of 24 months, the *Clostridium histolyticum* group was less abundant among children with ADHD. At the age of 13 years, there were no statistically significant differences in gut microbiota composition between children with or without neuropsychiatric disorders. The randomized trial did not present data about alpha-, beta-diversity and composition at class, order and family level.

Risk of bias

The assessment of risk of bias is presented in Supplementary Tables 2 and 3. As far as selection of ADHD patients and healthy controls (or exposed / unexposed cohort) is concerned, all studies complied with low risk of bias in the Newcastle—Ottawa Quality Assessment Scale (for case—control and cohort studies respectively). Some problems occurred in the comparability of the two groups, as only in two studies they were matched for all confounding factors. Finally, none of the studies gave information on non-responder rates.

DISCUSSION

This systematic review highlighted differences in gut microbiome features in association with ADHD occurrence and severity. Convergence of results between studies was observed while studying microbial genera. Aarts et al. (2017)[23] and Wan et al. (2020)[26] both agreed upon an increased abundance of *Odoribacter* in the ADHD group versus controls; Akram et al. (2017)[29] also reported a difference in the abundance of *Odoribacter*. An effect on neurotransmitter production and dopamine metabolism has been attributed to *Odoribacter*[31] which could potentially contribute to the occurrence of ADHD. *Odoribacter* contributes to the production of short-chain fatty acid (SCFA).[32] These acids have neuroactive and anti-inflammatory effects and in high levels have been shown to worsen ASD symptoms.[33,34] Similar effects have been hypothesized for ADHD.[33,35] Higher numbers of *Odoribacter* have also been associated with other neurodevelopmental disorders such as autism spectrum disorders.[34] On the other hand, in individuals with ADHD, a decrease of activity of the dopamine reward system has been established.[36]

Increased abundance of *Eggerthella* was found in ADHD according to two studies[20,23] and this bacterial genus has been linked to dopamine metabolism as well.[37] *Faecalibacterium* was found by three studies in decreased abundance in ADHD.[20,24,26] Low numbers of *Faecalibacterium* have been linked to inflammation through the release of cytokines, which have been reported to be higher in ADHD children[38,39] indicating another possible pathway to ADHD. *Faecalibacterium* has been also found decreased in adults suffering from psychiatric conditions, such as bipolar disorder and depression.[40,41] According to Jiang et al. (2018),[22] *Faecalibacterium* was negatively associated with ADHD severity and hyperactivity.

At the species level Wan et al. (2020)[26] remained consistent with the findings pertaining to bacterial phyla, reporting lower abundance of *Faecalibacterium prausnitzii* and higher of *Odoribacter splanchnicus* and thus strengthening the hypotheses mentioned above.

Another bacterial species found in greater abundance in the ADHD group, according to two studies[23,28] was *Bacteroides uniformis*. This particular species has been suspected of having a role in the development of parts of the brain, such as the frontal lobe and the hippocampal region.[42] In individuals with ADHD certain regions of the brain, such as prefrontal cortex, basal ganglia and parietal lobe show changes when compared to healthy controls.[43] Similar changes are observed in the connectivity between these regions in ADHD patients.[44] Wang et al. (2019)[28] suggested that increased frequency of *B.uniformis* could be related to ADHD susceptibility and Prehn-Kristensen (2018)[27] concluded that *Bacteroides species* levels correlated with levels of hyperactivity and impulsivity.

Regarding alpha diversity, no consistent differences arose between ADHD patients and healthy controls. The findings of two studies[20,21] indicated though that more information should be obtained; afterall according to Prehn-Kristensen et al. (2018)[27] levels of hyperactivity were significantly correlated with changes in alpha diversity. The results regarding b-diversity were also inconclusive, whereas only half the studies came up with results. Studies that examined[21,23,28] the microbial phyla did not seem to agree, so no safe conclusion can be derived at this point. Similarly, the results about differences in the gut microbiome at the order and family level were contradictory when comparing ADHD subjects versus healthy controls; nevertheless, two studies agreed upon an increase in abundance of the *Prevotellaceae* family in ADHD-C cases.[20,21]

Regarding the randomized trial by Pärtty et al. (2015)[22] it is interesting to mention that, although differences were noted up to the age of 24 months, by the time of the last examination of participants at the age of 13 years, there was no significant difference in the consistency of the gut microbiome between the children that were diagnosed with ADHD versus those that were not. (Table 3).

As deduced from the above mentioned results, a number of findings of the included studies do not seem to correlate or overlap. A few reasons could be suggested for this, such as different geographic regions, variations in age and different dietary habits of the participants.

Various limitations of this systematic review can be addressed. First, no quantitative synthesis (meta-analysis) was attempted, in view of the small number of eligible studies and marked heterogeneity in the reporting or results and indices across the individual published reports. In addition, the vast majority of the evidence stemmed from case-control studies; longitudinal cohort studies are needed to further validate the relevant hypotheses. Concerning the external validity of results, various world regions, such as Latin America, Australia and Africa were not represented in the body of evidence. Variability in the age groups and dietary habits should also be addressed by future studies as potential modifiers of the association between gut microbiome and ADHD.[45]

In conclusion, even correlations between gut microbiome features and manifestation of ADHD symptoms seem to emerge, with plausible mechanistic explanations, additional studies are needed to further investigate the interplay between intestinal microflora, ADHD occurrence and severity.

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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COMPETING INTERESTS

The authors declare no competing interests.

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