GUT MICROBES
2020, VOL. 11, NO. 5, 1172–1187
https://doi.org/10.1080/19490976.2020.1748258

Taylor & Francis
Taylor & Francis Group

REVIEW

OPEN ACCESS Check for updates

Gut microbiota profiles of autism spectrum disorder and attention deficit/hyperactivity disorder: A systematic literature review.

Caspar Bundgaard-Nielsen [a,b], Julie Knudsen [a,b], Peter D. C. Leutscher [a,b], Marlene B. Lauritsen [c], Mette Nyegaard [d], Søren Hagstrøm [a,b,e], and Suzette Sørensen [a,b]

[a]Centre for Clinical Research, North Denmark Regional Hospital, Aalborg, Denmark; [b]Department of Clinical Medicine, Aalborg University, Aalborg, Denmark; [c]Research Unit for Child and Adolescent Psychiatry, Aalborg University Hospital, Aalborg, Denmark; [d]Department of Biomedicine, Aarhus University, Aarhus, Denmark; [e]Department of Pediatrics, Aalborg University Hospital, Aalborg, Denmark

ABSTRACT

Accumulating evidence has implicated an involvement of the gut-brain axis in autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD), however with highly diverse results. This systematic review aims to describe and evaluate studies investigating the gut microbiota composition in individuals with ASD or ADHD and to evaluate if variations in gut microbiota are associated with these disorders.

Twenty-four articles were identified in a systematic literature search of PubMed and Embase up to July 22, 2019. They consisted of 20 studies investigating ASD and four studies investigating ADHD. For ASD, several studies agreed on an overall difference in β-diversity, although no consistent bacterial variation between all studies was reported. For ADHD, the results were more diverse, with no clear differences observed.

Several common characteristics in gut microbiota function were identified for ASD compared to controls. In contrast, highly heterogeneous results were reported for ADHD, and thus the association between gut microbiota composition and ADHD remains unclear. For both disorders, methodological differences hampered the comparison of studies.

ARTICLE HISTORY
Received 23 December 2019
Revised 4 March 2020
Accepted 21 March 2020

KEYWORDS
Autism Spectrum Disorder; attention-deficit hyperactivity disorder; neurodevelopmental disorders; microbiota; microbiome; systematic Review; gut-Brain axis

Introduction

In recent years, the prevalence of autism spectrum disorder (ASD) and attention-deficit disorder/attention-deficit/hyperactivity disorder (in this paper both disorders are referred as ADHD) has increased. Globally, ASD and ADHD are estimated to affect 1.0–2.0%[1,2] and 7.2%,[3] respectively, of all children and both disorders are associated with potentially severe social, adaptive, and educational problems. Thus, the development of these disorders is receiving increasing research attention.[4,5] While ASD describes a range of abnormalities characterized by impairment of social and communicative skills combined with restrictive-repetitive behavior, ADHD is defined by symptoms of inattention, impulsivity, and/or hyperactivity.[6] Despite these seemingly different symptoms, the two disorders are often co-existing, with previous studies reporting that up to 63% of ASD cases displayed ADHD symptoms.[6] Both disorders have substantial genetic contributions, with heritability estimates of approximately 54% and 74% for ASD and ADHD, respectively.[7,8] Furthermore, the two disorders share several genetic variants.[9] Despite these clear genetic involvements, heritability has not been able to satisfyingly predict the disorders, and instead, they are believed to be the result of a complex interaction between genetic and environmental factors.[7,8,]

For both ASD and ADHD, gastrointestinal (GI) symptoms are common, with constipation, diarrhea, and GI pain affecting up to 70% of ASD patients,[10,11] and the intensity of GI symptoms are positively correlated with ASD severity.[10,12] Similar to ASD, GI symptoms like constipation, fecal incontinence, and abdominal pain are commonly reported by ADHD patients.[13,14] Based on these observations, dietary interventions have been attempted. These include the use of gluten and casein-free diets for management of ASD symptoms,[15] and, for ADHD, omega-3, and −6 fatty acid supplementation and removal of food coloring. Results have varied, although reduction of core

CONTACT Caspar Bundgaard-Nielsen c.bundgaardnielsen@rn.dk Centre for Clinical Research North Denmark Regional Hospital and Department of Clinical Medicine, Aalborg University, Aalborg, Denmark Bispensgade 37, 9800 Hjoerring, Denmark
Supplemental data for this article can be accessed on the publisher's website.

symptoms of ASD and ADHD has been demonstrated.[15,16] Overall, while dietary interventions have not been successful in treating ASD or ADHD, the symptom improvements observed may suggest that components of the GI tract are involved in ASD or ADHD. A number of studies has suggested that the gut microbiota may serve as one of these components.[17–19]

The GI tract contains a thriving population of bacteria, that together with viruses, fungi, protozoa, and archaea, forms a community of microorganisms termed the gut microbiota.[20] Variations within the normal bacterial composition have been associated with the development of different pathophysiological conditions including type 2 diabetes,[21] obesity,[22] and inflammatory disorders.[23–25] Studies have indicated that GI bacteria are involved in a bidirectional interaction with the brain, which has been shown to be important for normal neurodevelopment.[26,27] Disruption of this interaction, termed the "gut-brain axis", has been hypothesized to be implicated in several neurological or psychiatric disorders like Parkinson's disease,[28] depression,[29,30] or bipolar disorder.[31] A number of direct and indirect contributing pathophysiological mechanisms has been proposed by which the gut microbiota may impact these disorders. Direct mechanisms include stimulation of the vagus nerve[32,33] and production of psychoactive metabolites as reported for ASD.[34] The indirect mechanisms include a number of functional differences that may result in increased GI tract permeability,[35] allowing leakage of bacterial products like lipopolysaccharides to the blood, and thus result in low-grade systemic inflammation.[28,]

Several studies have examined the role of gut microbiota in ASD using culturing or targeted approaches.[17,36,37] A recent preclinical study demonstrated that autism-like behavior could be transferred to mice through fecal microbiota transplant from children with ASD.[38] Other studies have attempted probiotic treatment, but with conflicting results.[39–41] Although gut microbiota has been suggested as a potential clinical target in treatment, the role of gut microbiota in ASD is still not completely understood.[42] Unlike ASD, information on the role of gut microbiota in ADHD is limited. In the few studies published so far, a lowered abundance of fecal *Bifidobacterium* in infancy or early life infection with *Streptococcus* has been associated with increased risk of developing ADHD.[18,43]

Despite several indications suggesting a relationship between an altered gut microbiota and ASD or ADHD, the nature of this involvement is still not clear. In order to facilitate the use of gut microbiota in improving diagnosis and treatment of core symptomology in ASD and ADHD, we require a better understanding of which bacteria are associated with these neurodevelopmental disorders, and how they affect their pathophysiological characteristics.

Thus, the aim of this study was to investigate and describe the current findings relating to altered gut microbiota composition in individuals with ASD and ADHD.

Methods

Search protocol

The protocol for this systematic review was registered at the International Prospective Register of Systematic Reviews (PROSPERO) under the ID number CRD42018111458, prior to commencement of this study. The guidelines provided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) were used.[44] A systematic search strategy was performed prior to July 22, 2019, using the databases PubMed and Embase, with no restrictions on publication year. Search strings were tailored for each database, based on existing publications, and are visualized in Table 1. The references of included studies were screened to identify potentially missed studies.

Eligibility criteria

Articles were included based on the following criteria: The included studies must be original studies performed in humans, diagnosed with one or both of the following diagnoses: ASD (299.00 or 299.80 according to the "Diagnostic and Statistical Manual of Mental Disorders" (DSM)-IV or 5 criteria and F84.0, F84.1, F84.5, or F84.8 according to the "International Statistical Classification of Diseases and Related Health Problems" (ICD)-10 criteria) or ADHD (314.00 or 314.01 according to DSM-IV or 5 or F90.0, F90.1 or F98.8 according to the ICD-10 criteria). The complete microbial community must be assessed in fecal samples. The microbial community should be compared to

Table 1. Search terms used for the systematic search. Use of "AND" or "OR" in the search engines has been indicated.

	Horizontal lines divided by "AND"	
	Cases	Outcome
Search terms (Vertical lines divided by "OR")	• Neurodevelopmental disorders[MESH] • Attention Deficit Disorder*[Text Word] • Attention Deficit Hyperactivity Disorder[Text Word] • ADHD[Text Word] • ADD[Text Word] • Autism[text word] • "Autism Spectrum Disorder"[MESH] • Neurodevelopmental*[text word] • Neurodevelopmental disorder[MESH]	• (Microbiology[MESH] OR Microbiology[Subheading] OR Microbiology[Text Word])AND(Feces"[MESH] OR Gastrointestinal Tract[MESH]) • Gastrointestinal Microbiome[MESH] • Gastrointestinal Microbiome*[text word] • Gastrointestinal Microbiot*[text word] • Gut microbiot*[text word] • Gut microbiome*[text word] • Intestinal Microbiot*[text word] • Intestinal Microbiome*[text word].

a control group without either ASD or ADHD. The articles must be written in English or Danish.

Articles were excluded if they included less than 10 study participants or focused on co-morbidity between ASD/ADHD and other disorders.

All inclusion or exclusion criteria are available at the Collaborative Approach to Meta Analysis and Review of Animal Data from Experimental Studies (CAMARADES) website SyRF (http://app.syrf.org.uk/projects/5ffc6aab-3415-43b3-be6a-1fc5084f08fa/detail).

Study selection

Articles obtained from the literature searches were combined, and duplicates were removed using the automatic function implemented in the reference manager Mendeley (https://www.mendeley.com/). The articles were analyzed in two stages: Initially, titles and abstracts were screened independently by two researchers (CBN and JKK), using SyRF (http://syrf.org.uk/), according to the eligibility criteria. Next, the included articles were subjected to whole-paper revision. Disagreements between the two reviewers were resolved by consensus-based discussion, and, if necessary, a third reviewer was involved (SES).

Data extraction and quality assessment

Data were extracted to a Microsoft Excel file (Supplementary Table 1), focusing on demographics, diagnostic methodology, microbiota assessment methodology, bacterial richness, diversity, and taxonomic bacterial composition (phylum, family, genus, and species only). Meta-analysis was not performed due to the heterogeneity of methodology.

Quality assessment of the included studies was evaluated, using the Newcastle-Ottawa Scale (NOS) for case–control studies. NOS contains three criteria: selection (are cases and controls effective community controls?), comparability (are cases and controls comparable?), and exposure (how are diagnosis and microbiota assessed?). A quality score ranging from 0 to 10 was obtained by the use of a rating algorithm previously described:[42] 0–5 (poor), 6–7 (moderate), and 8–10 (high).

Results

Study selection

The initial database search generated 1,841 articles, which were reduced to 1,532 unique articles after automatic duplicate removal. Subsequent screening of titles and abstracts resulted in 62 articles assigned to whole paper revision. During whole paper revision, 38 articles were excluded due to non-complete eligibility criteria upon closer inspection. This included articles that only investigated a subset of the gut microbiota (n = 12); were conference abstracts (n = 12); characterized gut microbiota in GI biopsies or urine samples rather than fecal samples (n = 5); only characterized gut microbiota following pro- or prebiotic intervention (n = 4); were duplicates of already included studies (n = 2); had less than 10 study participants (n = 2); or did not compare the gut microbiota to a control population (n = 1). Finally, 24 original articles were included in this systematic review. These articles included 20 articles investigating ASD[45-64] and 4 investigating ADHD[65-] (Figure 1, supplementary data 1). None

of the studies included study participants with both disorders. [Figure 1 near here]

As indicated in Table 2, all studies received a NOS score ranging from six (moderate) to eight (high). Four studies,[47,52,54,57] all investigating ASD, received a score of eight (high), due to matching cases and controls on other variables than age alone. Conversely, four studies (three investigating ASD and one investigating ADHD)[49,51,64,65] received a score of six (moderate), due to inadequate description of samples,[64] controls represented by children undergoing surgery and thus not being representative community controls,[49] or controls being older than ASD or ADHD cases (tables 3 and 4).[51,65] The remaining studies all received a score of seven (moderate).

Characteristics of studies investigating ASD or ADHD

Demographics of the included studies are seen in tables 3 and 4 for ASD and ADHD, respectively. Geographically, the studies were performed in USA,[45,47,58,59,61,63,64] Europe,[49,51,56,57,60,65,66] Taiwan, People's Republic of China,[48,50,52-55,] Australia,[46] and India.[62] The studies investigating ASD included in total 733 cases and 590 controls (138 siblings and 452 non-related controls), whereas the studies of ADHD included in total 114 cases and 156 controls (21 siblings and 135 non-related controls).

The majority of studies used non-related participants as controls, while four studies compared ASD cases to siblings,[46,57,59,62] and two (one ASD and one ADHD) compared cases to both siblings and non-related controls.[45,65] It is noteworthy, that while the majority of studies included cases and controls younger than 18 y only, one study investigating ADHD[65] included cases and controls older than 18 y. For most studies investigating ASD, there was a higher percentage of males among cases compared to controls (total of 74.6% for cases, 41.3% for siblings, and 63.3% for non-related controls, for all studies providing this). This was also true for studies

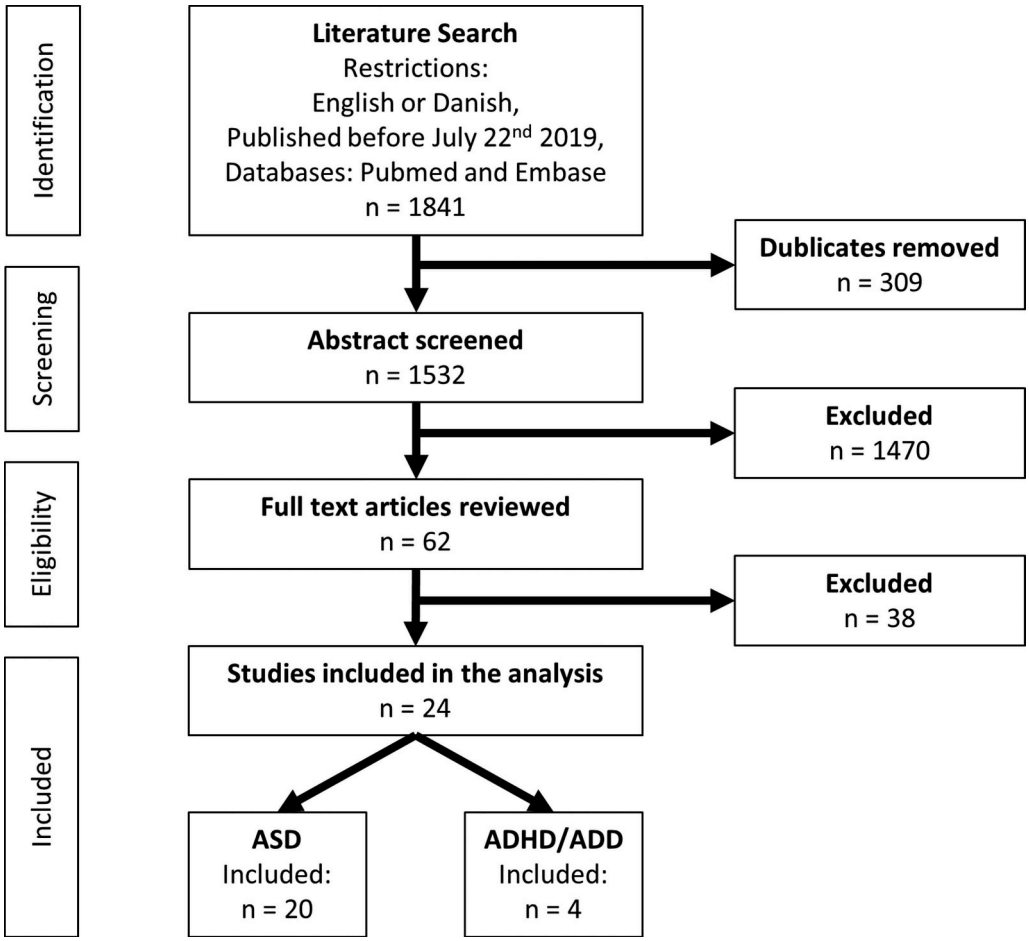

Figure 1. PRISMA flow diagram, summarizing the studies identified during the systematic literature search and reviewing process.

Table 2. Quality assessment of included studies based on the newcastle-ottawa scale for case–control studies. The articles were rated based on selection and characterization of cases and controls (Selection, max score 4), comparability between case and controls (Comparability, max score 2), and ascertainment of effects of microbiota (Exposure, max score 4), for a potential score ranging from 0 to 10 points.

Study	Year	Selection	Comparability	Exposure	Total
ASD					
Finegold et al.[45]	2010	4	1	2	**7**
Gondalia et al.[46]	2012	4	1	2	**7**
De Angelis et al.[57]	2013	4	2	2	**8**
Kang et al.[58]	2013	4	1	2	**7**
Son et al.[59]	2015	4	1	2	**7**
Strati et al.[60]	2017	4	1	2	**7**
Kang et al.[61]	2017	4	1	2	**7**
Pulikkan et al.[62]	2018	4	1	2	**7**
Kang et al.[63]	2018	4	1	2	**7**
Berding et al.[64]	2018	3	1	2	**6**
Rose et al.[47]	2018	4	2	2	**8**
Zhang et al.[48]	2018	4	1	2	**7**
Coretti et al.[49]	2018	3	1	2	**6**
Li et al.[50]	2019	4	1	2	**7**
Carissimi et al.[51]	2019	4	0	2	**6**
Liu et al.[52]	2019	4	2	2	**8**
Zhai et al.[53]	2019	4	1	2	**7**
Ma et al.[54]	2019	4	2	2	**8**
Wang et al.[55]	2019	4	1	2	**7**
Plaza-Díaz et al.[56]	2019	3	2	2	**7**
ADHD					
Aarts et al.[65]	2017	4	0	2	**6**
Prehn-Kristensen et al.[66]	2018	4	1	2	**7**
Jiang et al.	2018	4	1	2	**7**
Wang et al.	2019	4	1	2	**7**

investigating ADHD (total of 77.2% for cases compared to 62.8% for controls). Only three studies used gender-matched cases and controls.[52,54,66] We also recorded information regarding the use of special diets or nutritional supplements, presence of GI symptoms as well as the use of medication. We found that for three studies investigating ASD[45,58,59] and one study investigating ADHD, the diet, or use of probiotics, of cases differed from that of controls. Similarly, presence of GI symptoms (primarily constipation, but also diarrhea and abdominal pain) was common for ASD (reported in 31.5% of cases versus and 7.3% for controls), which was not seen for ADHD. All studies excluded participants who recently received antibiotics, while two studies investigating ADHD[65,66] included cases that received ADHD medication. No other medical treatments were observed to be prevalent in the studies (tables 3 and 4).

Handling and analysis of samples

A number of differences in sample handling and analyzes was observed between the individual studies, and are described in Table 5. Following sample collection, the majority of studies stored the fecal samples at either −20°C or −80°C, while other studies used preserving buffers[47,50,57,59] or stored samples at 4°C.[54,65,66] Two studies did not provide information on storage of samples.[51,58] For DNA extraction, most studies used commercial spin column-based extraction kits, with approximately half of the studies implementing pretreatment steps to increase DNA extraction from gram-positive bacteria.[47,50,53,56-61,63,66,] All studies, with the exception of two that used metagenomic sequencing,[51,55] assessed fecal microbiota using amplicon sequencing of the 16 S ribosomal ribonucleic acid (rRNA) gene, targeting a number of hypervariable regions. Taxonomy was assessed using a variety of different databases, with Greengenes[47-50,60–64] being the most common.

Children and adolescents with ASD have distinct gut microbiota

The gut microbial communities of ASD cases were compared to controls, assessing α- and β-diversity as well as changes in individual bacterial abundances.

Table 3. Demographics of ASD cases included in this systematic review. No studies included participants that received antibiotic treatment. In the row labeled "Total", the total number of participants, and total gender distribution and GI symptoms percentage (for studies providing numbers) for all studies combined, are displayed.

		ASD						
Study	Country	Sample size		Gender (Male %)	Age (years)	Diagnostic instrument	GI symptoms (% of total)	Special diet
Finegold et al.[45]	USA	ASD:	33	72.7	2-13	N/A	100.0	Diet: Unspecified number of cases used special diet.
		SIB:	7	28.6			0.0	
		Ctrl:	8	62.5			0.0	
Gondalia et al.[46]	Australia	ASD:	51	82.4	2-12	N/A	54.9	N/A
		SIB:	53	35.8			7.5	
De Angelis et al.[57]	Italy	ASD:	20	46.7	4-10	ADI-R, ADOS	0.0	No special diet
		SIB:	10				0.0	
Kang et al.[58]	USA	ASD:	20	90.0	3-16	ADI-R, ADOS,	100.0	Diet: 5 cases.
		Ctrl:	20	85.0		ATEC,	0.0	Dietary supplements: 13 cases + 8 Ctrls.
						PDD-BI		
Son et al.[59]	USA	ASD:	59	88.1	7-14	N/A	42.4	Diet: 4 cases + 1 Ctrl.
		SIB:	44	47.7		(DSM-IV)	29.5	
Strati et al.[60]	USA	ASD:	40	77.5	Mean	N/A	12.5	No special diet
		Ctrl:	40	70.0	age:	(DSM-IV)	27.5	
					11.1			
Kang et al.[61]	Italy	ASD:	18	88.9	7-16	ADI-R	100.0	No special diet
		Ctrl:	20	90.0			0.0	
Pulikkan et al.[62]	India	ASD:	30	93.3	3-16	CARS,	Common for	No special diet
		SIB:	24	62.5		ISAA	ASD cases	
Kang et al.[63]	USA	ASD:	2123	65.2	4-17	ATEC,	Common for	N/A
		Ctrl:		95.6		PDD-BI	ASD cases	
Berding et al.[64]	USA	ASD:	26	73.1	2-7	N/A	Common for	No special diet
		Ctrl:	32	59.4			ASD cases	
Rose et al.[47]	USA	ASD:	50	84.0	<13	ADI-R, ADOS	42.0	No special diet
		Ctrl:	41	92.7			17.1	
Zhang et al.[48]	People's	ASD:	35	82.9	3-8	N/A	31.4–60.0	No special diet
	Republic of	Ctrl:	6	83.3		(DSM-IV)	0.0	
	China							
Coretti et al.[49]	Italy	ASD:	11	81.8	2-4	ADOS2,	18.2	No special diet
		Ctrl:	14	57.1		ADI-R, GMDS,	0.0	
						VABS, CARS		
Li et al.[50]	People's	ASD:	59	84.7	2-10	ADOS, ABC	50.8	No special diet
	Republic of	Ctrl:	30	66.7			23.3	
	China							
Carissimi et al.[51]	Italy	ASD:	16	100.0	2-6	GMDS, ADOS2	Common for	N/A
		Ctrl:	7	28.6	5-16		ASD cases	
Liu et al.[52]	People's	ASD:	30	83.3	2.5-18	N/A	30.0	No special diet
	Republic of	Ctrl:	20	80.0		(DSM-5, ICD-10)	5.0	
	China							
Zhai et al.[53]	People's	ASD:	78	71.8	Mean	ATEC	Common for	N/A
	Republic of	Ctrl:	58	53.4	age:		ASD cases	
	China				4.9			
Ma et al.[54]	People's	ASD:	45	86.7	6-9	CARS	N/A	No dietary differences between cases and controls
	Republic of	Ctrl:	45	86.7				
	China							
Wang et al.[55]	People's	ASD:	43	83.7	2-8	N/A	44.2	No difference between cases and controls
	Republic of	Ctrl:	31	58.1		(DSM-5)	0.0	
	China							
Plaza-Díaz et al.[56]	Spain	ASD:	48	Matched	2-6	ADI-R, ADOS,	Common for	N/A
		Ctrl:	57			PDD-BI	ASD cases	
Total		**ASD**	**733**	**74.6**	**2-18**		**31.5**	
		SIB	**138**	**41.3**			**12.3**	
		Ctrl	**452**	**63.3**			**5.8**	

The most consistent microbiota differences are visualized in Table 6, with a more comprehensive list presented in supplementary data 1. Highly heterogeneous results were obtained for α- (number of species and their diversity within samples) and β-diversity. (difference in bacterial composition between

Table 4. Demographics of ADHD cases included in this systematic review. No studies included participants that received antibiotic treatment. In the row labeled "Total", the total number of participants, and total gender distribution and GI symptoms percentage (for studies providing numbers) for all studies combined, are displayed.

Study	Country	Sample size		Gender (Male %)	Age (years)	Diagnostic instrument	GI symptoms (% of total)	Special diet or ADHD medication
Aarts et al.[65]	The Netherlands	ADHD: SIB: Ctrl:	19 21 56	68.4 SIB/ctrl: 53.2	Mean age: ADHD: 19.5 SIB+Ctrl: 27.1	K-SADS-PL	N/A	Diet: N/A Unspecified number of cases received ADHD medication
Prehn-Kristensen et al.[66]	Germany	ADHD: Ctrl:	14 17	100.0 100.0	Mean age: 11.9	K-SADS-PL	N/A	Diet: No difference in diet. 10 cases received Methylphenidate.
Jiang et al.	People's Republic of China	ADHD: Ctrl:	51 32	74.5 68.8	6-10	K-SADS-PL	0.0 0.0	No special diet No pharmacological treatment of ADHD
Wang et al.	Taiwan	ADHD: Ctrl:	30 30	76.7 60.0	6-16	K-SADS-PL ADHD-RS	0.0 0.0	Diet of cases differed from that of controls. No pharmacological treatment of ADHD
Total		**ADHD** **SIB** **Ctrl**	**114** **21** **135**	**77.2** **SIB/ctrl** **62.8**	**6-N/A**		**N/A/** **0.0**	

samples). The majority of studies did, however, find that the overall microbiota composition of ASD cases differed from that of controls.[45,47-50,52-54,57,60,63,64]

A number of differences was observed between ASD cases and controls when comparing the relative abundance of individual bacterial phyla and genera. For phyla, three studies reported increased relative abundance of *Proteobacteria* in ASD cases.[45,49,56] Nine studies reported altered *Firmicutes-Bacteroidetes* ratio, although they differed in the direction of change.[45,48,49,52,53,57,60,62,64] For bacterial genera, several studies reported increased relative abundance of *Bacteroides*,[45,49,53,57] *Barnesiella*,[52,57,63] *Clostridium*,[50,55,57,64] and *Roseburia*,[49,57,64] as well as reduced relative abundance of *Bifidobacterium*,[45,49,57,61,64] *Coprococcus*,[57,58,63] *Dialister*,[45,60,64] *Faecalibacterium*,[57,63,64] *Prevotella*,[50,57,58,63] and *Streptococcus*[45,48,49,52,57] in cases. However, no specific bacteria consistently differed between ASD cases or controls in all of the included studies. A few of the studies also looked at the effects of microbial differences; The metabolism was reported to be affected by several of the microbial changes associated with ASD.[48,49,51,52,54,55,57,64] This was especially true for short-chain fatty acids (SCFAs) metabolism, that was reported to be affected by changes in *Faecalibacterium, Ruminococcus*, and *Bifidobacterium* composition.[48,49,52,57,64] Further more, two studies reported that the gut microbiota of ASD was associated with increased concentrations of pro-inflammatory cytokines,[47,51] while Wang et al. reported that the increased *Clostridium* and *Bacteroides* associated with ASD resulted in reduced cortisol concentrations.

Studies investigating gut microbiota in ADHD cases yield inconclusive results

As for ASD, studies investigating the gut microbiota of ADHD compared to controls used α- and β-diversity as well as changes in individual bacterial abundances (Table 6, more comprehensive list in supplementary data 1). No clear overall conclusion could be drawn from the studies. The two studies originating from Europe observed that the gut microbiota β-diversity of ADHD cases differed from controls,[65,66] whereas none of the two East-Asian studies observed any significant differences.' Furthermore, changes in individual bacteria were inconsistent between the four studies. All found ADHD specific changes, but no studies agreed on what bacterial taxa differed. Three studies discussed the causes and effects of the gut microbial variations. Wang et al. reported increased *Bacteroides* in children with ADHD, which was correlated to dietary differences. Jiang et al. reported that *Faecalibacterium* was negatively associated with ADHD symptoms, while Aarts et al.[65] reported that genes encoding cyclohexadienyl dehydratase (CDT) had increased functionality in the ADHD-associated bacteria. The authors

Table 5. Handling of samples from cases and controls. N/A: No information provided.

Study	Sample storage	DNA extraction	Sequencing technique/target	Reference database
ASD				
Finegold et al.[45]	Transported overnight on ice	QIAamp DNA Stool mini kit	454 FLX pyrosequencing, 16 S rRNA	Custom database similar to RDP-II
Gondalia et al.[46]	Transported overnight on ice	QIAamp DNA Stool mini kit	454 FLX pyrosequencing, 16 S rRNA V1-V3 region	BLASTn
De Angelis et al.[57]	RNAlater, frozen at −80°C	Bead-beating. FastDNA pro soil-direct kit	454 FLX pyrosequencing, 16 S rRNA V1-V3 region	GenBank
Kang et al.[58]	−20°C for up to 24 hours	Bead-beating. QIAamp DNA Stool mini kit	454 FLX pyrosequencing, 16 S rRNA V2-V3 region	SSURef
Son et al.[59]	RNAlater, stored cold overnight	Bead-beating. ZR Fecal DNA MiniPre	Illumina sequencing, 16 S rRNA V1-V2 + V1-V3 region	SILVA
Strati et al.[60]	−80°C	Bead-beating. FastDNA Spin kit for feces	454 FLX pyrosequencing, 16 S rRNA V3-V5 region	Greengenes
Kang et al.[61]	N/A	Bead-beating. Powersoil DNA kit	Illumina Miseq, 16 S rRNA V4 region	Greengenes
Pulikkan et al.[62]	−80°C	QIAamp DNA Stool mini kit	Illumina sequencing, 16 S rRNA V3 region	Greengenes
Kang et al.[63]	−20°C for up to 24 hours	Bead-beating. Powersoil DNA kit	454 FLX pyrosequencing, 16 S rRNA V2-V3 region	Greengenes
Berding et al.[64]	−80°C	QIAamp Fast DNA Stool mini kit	Illumina sequencing, 16 S rRNA V2-V3 region	Greengenes
Rose et al.[47]	RNAlater, frozen at −20°C	Bead-beating. Powersoil DNA kit	Illumina sequencing, 16 S rRNA V3-V4 region	Greengenes
Zhang et al.[48]	−80°C within few hours	N/A	Illumina sequencing, 16 S rRNA V3-V4 region	Greengenes
Coretti et al.[49]	−80°C	QIAamp DNA Stool mini kit	Illumina Miseq, 16 S rRNA V3-V4 region	Greengenes
Li et al.[50]	99% ethanol. Later frozen at −80°C	Bead-beating. FastDNA Spin kit for feces	Illumina Hiseq, 16 S rRNA V1-V2 region	Greengenes
Carissimi et al.[51]	N/A	QIAamp DNA Stool mini kit	Illumina paired end Shotgun sequencing	-
Liu et al.[52]	−80°C within 30 min	QIAamp Fast DNA Stool mini kit	Illumina Miseq, 16 S rRNA V3-V4 region	SILVA
Zhai et al.[53]	Transported on ice	Bead-beating. FastDNA Spin kit for soil	Illumina Miseq, 16 S rRNA V3-V4 region	N/A
Ma et al.[54]	4°C for up to 12 hours	QIAamp Fast DNA Stool mini kit	Illumina Hiseq, 16 S rRNA V3-V4 region	SILVA
Wang et al.[55]	−80°C upon delivery at lab	StoolGen fecal DNA extraction kit	Illumina Hiseq Shotgun sequencing	-
Plaza-Díaz et al.[56]	−80°C upon delivery at lab	95°C pretreatment in lysis buffer. QIAamp DNA Stool mini kit	Illumina Miseq, 16 S rRNA V3-V4 region	RDP
ADHD				
Aarts et al.[65]	Stored at 4°C for up to 24 hours	Dneasy blood and tissue kit	454 FLX pyrosequencing, 16 S rRNA V3-V6 region	RDP
Prehn-Kristensen et al.[66]	Stored at 4°C	Bead-beating. FastDNA Spin kit for Soil	Illumina Miseq, 16 S rRNA V1-V2 region	N/A
Jiang et al.	−20°C for up to 24 hours	QIAamp DNA Stool mini kit	Illumina Miseq, 16 S rRNA V3-V4 region	N/A
Wang et al.	−20°C for up to 24 hours	Pretreatment with lysis buffer. QIAamp DNA Stool mini kit	Illumina Miseq, 16 S rRNA V3-V4 region	RDP

further reported that the increased abundance of CDT was significantly associated with decreased reward anticipation, previously reported in ADHD.[65]

Discussion

Understanding the microbial communities associated with ASD and ADHD has the potential of improving current treatment options for individuals with these disorders. While studies have attempted to utilize fecal microbiota transfer[61] or probiotics in the treatment of ASD or ADHD, results have been limited.[18,39,61] Given the large inter-individual variations in the healthy microbiome,[67] a better understanding of normal variation, as well as whether gut bacteria are involved in the etiology of ASD and

Table 6. Table depicting the most important observations on bacterial composition between ASD or ADHD cases, and controls. Only bacteria, for which two separate studies have agreed on the direction of difference, are displayed in the table. Empty boxes represent that no difference was reported for this measurement, between cases and controls in the represented study. For De Angelis et al.[57] autism and PDD-NOS were combined. ↑ = higher α diversity or bacteria are more abundant, in ASD/ADHD compared to control; ↓ = Lower α diversity or bacteria are less abundant, in ASD/ADHD compared to control; D = bacterial β-diversity differ between ASD or ADHD cases compared to controls. N = No difference in β-diversity. – = no information.

	ASD																					ADHD						
Studies	49	50	61	62	63	64	65	66	67	68	51	52	53	54	55	56	57	58	59	60	**Total** ↑ ↓	69	70	71	72	**Total** ↑ ↓
α-diversity																										
Richness	↓		↑	↓					↓					↑			↑	↓	↓		3 5				↑	1 0
Diversity			↑	↓			↓		↓			↓	↑		↓	↓	↑	↓			3 7		↓		↑	1 1
β-diversity	D	N	D	N	N	D	N	N	D	D	D	D	D	D	-	D	D	D	-	-	D=12	D	D	N	N	D=2
Phylum																										
Actinobacteria	↓												↓						↑	↑	2 2	↑				1 0
Bacteroidetes	↑		↑			↓		↓		↓		↑	↑				↑				5 3					0 0
Firmicutes	↓		↓			↑		↑		↑		↓				↓	↓				3 5					0 0
Proteobacteria	↑												↑							↑	3 0					0 0
Genera																										
Actinomyces												↓	↓								0 2					0 0
Bacillus																	↑			↑	2 0					0 0
Bacteroides	↑		↑										↑				↑				4 0		↑			1 0
Barnesiella			↑						↑							↑					3 0					0 0
Bifidobacterium	↓		↓				↓	↑		↓			↓							↑	2 5	↑				1 0
Butyricimonas										↓		↑								↑	2 1					0 0
Clostridium	↓		↑							↑				↑					↑		4 1					0 0
Collinsella	↓		↑			↑															2 1					0 0
Coprococcus			↓	↓					↓				↑								1 3					0 0
Dialister	↓					↓				↓											0 3			↓		0 1
Dorea			↑			↑															2 0					0 0
Enterococcus																↑				↑	2 0					0 0
Escherichia			↓									↓									0 2					0 0
Eubacterium			↓													↓					0 2					0 0
Faecalibacterium			↓						↓	↓			↑								1 3			↓		0 1
Lachnospira			↓										↑					↓			1 2					0 0
Lactobacillus						↑		↑													2 0				↓	0 1
Megaspaera								↑						↑							2 0					0 0
Oscillospira			↓										↑				↑				2 1					0 0
Parabacteroides	↑		↓			↓							↑				↑				3 2		↓			0 1
Prevotella			↓	↓					↓					↓						↑	1 4		↓			0 1
Pseudomonas														↑		↑					2 0					0 0
Roseburia			↑							↑			↑								3 0					0 0
Ruminococcus	↓		↑										↑								2 1					0 0
Streptococcus	↓		↓									↓	↓	↑		↓					1 5					0 0
Sutterella												↑					↑				2 0					0 0
Turicibacter	↓		↓																		0 2					0 0
Veillonella						↓						↓									0 2					0 0

ADHD, is needed to develop future microbiota-based treatments.

Gut microbiota of ASD and/or ADHD

In this systematic review, we sought to evaluate whether individuals with ASD or ADHD had a distinct microbiota composition compared to controls. Importantly, for ASD, the majority of studies identified that the gut microbiota of ASD cases differed from controls, although no specific bacteria was consistently altered across studies. As suggested by Turnbaugh et al.,[68] the microbiome of a pathologic condition can also be defined by an altered function rather than an altered bacterial composition. Amongst bacteria reported to have increased relative abundance in ASD cases, several genera has previously been associated with inflammation.[69–71] Conversely, several commensal bacteria with lower relative abundance are known to induce anti-inflammatory effects[72,73] or are involved in the maintenance of normal metabolism.[64,73–75] The findings have been supported by Rose et al.[47] and Carissimi et al.,[51] who reported that ASD cases had an increased concentration of pro-inflammatory cytokines. However, we still lack more in-depth analyzes in the functions affected by the gut microbiota in ASD. These include, but are not limited to, studies investigating bacterial metabolites and effects on inflammation and metabolism.

Compared to ASD, the number of published studies investigating the involvement of gut microbiota in ADHD are surprisingly limited. This is supported by a recent systematic review, where only two studies on ADHD and gut microbiota were identified; both studies are also included in the present review.[76] Amongst the included studies, the results were furthermore too heterogeneous to make confident conclusions regarding whether ADHD is associated

with a different gut microbiota profile. Reduced relative abundances were reported of the bacterial genera *Parabacteroides, Prevotella*,[66] *Faecalibacterium, Dialister*, and *Lactobacillus*[77] in ADHD cases compared to controls. These genera are known to assist with maintenance of the normal GI tract function,[72,75,78-80] which fits with the observed functional differences in carbohydrate and fat metabolism in ADHD, reported by Wang et al. Both Aarts et al.[65] and Jiang et al. reported a significant correlation between specific microbial differences and ADHD symptomology. While intriguing, more studies are urgently needed to further elucidate whether these microbial interactions might directly influence the pathophysiology of ADHD. A previous study by Cheng et al.[81] further reported that single nucleotide polymorphisms (SNPs) associated with the genus *Desulfovibrio* and the order *Clostridiales*, were enriched in ADHD cases, although we could not substantiate this observation. Interestingly, in a large study of microbiota–drug interactions, Zimmermann et al.[82] reported that certain gut bacteria could chemically modify the common ADHD drug Methylphenidate. Since the response of ADHD patients to medication differs, studies are needed to investigate whether gut microbiota could be used to predict drug response in ADHD patients.

As previously mentioned, there is a high degree of overlap between ASD and ADHD. It is therefore interesting if the two disorders share gut microbiota variations. Both ASD and ADHD are associated with a lower relative abundance of commensal bacteria related to the maintenance of a healthy GI function, which may explain the high frequency of GI dysfunctional conditions. It is however important to note that the differences in methodologies and the reported heterogeneous microbiota compositions in the reviewed articles hamper our ability to investigate the possibility of a shared gut microbiota in ASD and ADHD.

Differences in methodology may explain the heterogeneous results

It is well known that several factors may have an influence on the composition of gut microbiota, including geographic, cultural, dietary, and demographical differences,[67,83-86] which may explain some of the observed discrepancies between different studies. Intriguingly, Winglee et al. showed that urbanized Chinese people had gut microbiota with closer resemblance to Americans rather than that of rural Chinese people.[87] This indicates that differences previously attributed to ethnical or geographical differences may instead be explained by differences in lifestyle. While a fiber-rich, plant-based diet is associated with a gut microbiota rich in the *Bacteroidetes* phylum and the genus *Prevotella*, a typical western diet is associated with increased *Firmicutes* and *Bacteroides*.[83,84,87,88] It is recognized, that children with ASD often have a lower vegetable intake compared to children without ASD, often due to selective eating and sensory disturbances,[89,90] and it is thus interesting, that several studies included in this systematic review reported increased *Bacteroides* and decreased *Prevotella* for ASD cases.[45,49,53,57,58,63]

GI dysfunctions, primarily constipation, and diarrhea, were common amongst the ASD cases in several of the studies included in this systematic review. As reported by Vandeputte et al.,[91] gut microbiota composition is highly associated with colon transit time as indicated by fecal consistency. While a fast transit time selects for fast-growing bacteria, the slow transit time observed in constipation enables more slowly growing bacteria to thrive. As a result, the increased presence of GI symptoms in ASD cases may explain some of the differences in gut microbiota observed between the studies.

Importantly, we observed that studies differed in selection of control groups. The majority of studies compared cases with non-related controls, some compared to siblings, and some to both groups, to correlate for similarities in environment. Finegold et al.[45] reported that the gut microbiota of siblings to children with ASD, had a bacterial composition resembling a middle group between ASD and non-related controls. This may explain why two studies using siblings as controls only observed none to minor bacterial differences compared to controls.[46,59]

While sequencing enables highly sensitive determination of the microbiota composition, several factors in conjunction with handling of samples may influence data output.[92,93] Among the studies included in this systematic review, several different storage techniques were utilized, ranging from lowered temperature to the use of storage buffers. While gut microbiota is robust, differences in storage can lead to growth or disruption

of susceptible bacteria, and thus result in differences in bacterial composition between studies.[92,94,95] Extraction of DNA from gram-positive bacteria is problematic, due to the presence of a thick cell wall, that can prevent effective bacterial lysis during DNA extraction.[93,96] This can lead to underrepresentation of gram-positive bacteria in studies investigating gut microbiota.[93] Only half the studies included in this systematic review took steps to increase DNA extraction from gram-positive bacteria. Despite this, we did not detect a clear pattern in differences in bacteria known to be difficult to extract, like the *Streptococcus* genera,[96] and the impact is thus uncertain. Finally, most of the included studies investigated microbiota composition by sequencing the different hypervariable regions of the 16 S rRNA gene. However, primers targeting different regions have different affinities to specific bacteria, and thus may capture different bacteria in the same samples.[93] This makes comparison of studies using primers targeting different regions problematic.

Besides differences in sample handling, the studies also differed in the choice of bioinformatics pipelines and reference databases. Two commonly used reference databases amongst the included studies were Greengenes (http://greengenes.secondgenome.com/) and SILVA (https://www.arb-silva.de/). As reported by Park et al.[97] these reference databases may not always identify the same microbial genera, which impairs proper comparison of studies. Here it is noteworthy that none of the included studies using the SILVA reference database identified differences in the *Bacteroidetes* phylum. In contrast, five out of nine studies using the Greengenes database reported differences for this phylum.

Overall, several methodological differences were observed between the studies included in this systematic review, but no single factor explained the heterogeneity. It is thus unclear whether the heterogeneous gut microbiota compositions for each disorder presented in this systematic review, represent natural variations, or whether several factors together cause these variations in gut microbiota.

Limitations

A number of limitations needs to be addressed: First, analysis of the included studies proved complicated, since they varied widely regarding methodology and demography. This made the performance of a meta-analysis unfeasible. Secondly, all systematic reviews are susceptible to publication bias, where studies reporting differences in microbiota composition between cases and controls are more likely to be published. We read the references of the included studies, to determine if other studies were missed in the systematic search. This did not reveal any additional studies, suggesting that we adequately covered the published literature. Finally, new studies may have been missed, if MESH terms had not been assigned at the time of the systematic search.

Conclusion

This systematic review has demonstrated that ASD and ADHD cases are associated with a gut microbiota different from controls without neurodevelopmental disorders. However, studies varied widely concerning methodology, resulting in highly heterogeneous gut microbiota compositions between studies. A specific ASD or ADHD-associated gut microbiota could therefore not be established, although, for ASD, a few shared functional differences were suggested. Future studies should consider investigating differences in gut microbiota function as well as composition. Furthermore, the differences in methodology and demography could have influenced the gut microbiota of the studies, and thus studies are needed that investigate the gut microbiota jointly in these often comorbid diagnoses.

Disclosure:

The authors declare no conflicts of interest.

Funding

This work was funded by the North Denmark Regional Hospital.

ORCID

Caspar Bundgaard-Nielsen http://orcid.org/0000-0002-2700-2378
Julie Knudsen http://orcid.org/0000-0002-5528-3592
Peter D. C. Leutscher http://orcid.org/0000-0003-1168-4041
Marlene B. Lauritsen http://orcid.org/0000-0001-7284-9255

Mette Nyegaard http://orcid.org/0000-0003-4973-8543
Søren Hagstrøm http://orcid.org/0000-0002-1217-808X
Suzette Sørensen http://orcid.org/0000-0002-4201-1168

References

1. Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, Kurzius-Spencer M, Zahorodny W, Robinson Rosenberg C, White T, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States. Surveill Summ. 2018;67:1–23.
2. Kim YS, Leventhal BL, Koh Y-J, Fombonne E, Laska E, Lim E-C, Cheon K-A, Kim S-J, Kim Y-K, Lee H, *et al.* Prevalence of autism spectrum disorders in a total population sample. Am J Psychiatry. 2011;168:904–912. doi:10.1176/appi.ajp.2011.10101532.
3. Thomas R, Sanders S, Doust J, Beller E, Glasziou P. Prevalence of attention-deficit/hyperactivity disorder: a systematic review and meta-analysis. Pediatrics. 2015;135:e994–1001. doi:10.1542/peds.2014-3482.
4. Knüppel A, Telléus GK, Jakobsen H, Lauritsen MB. Characteristics of young adults with autism spectrum disorder performing different daytime activities. J Autism Dev Disord. 2019;49:542–555. doi:10.1007/s10803-018-3730-7.
5. Scandurra V, Emberti Gialloreti L, Barbanera F, Scordo MR, Pierini A, Canitano R. Neurodevelopmental disorders and adaptive functions: a study of children with autism spectrum disorders (ASD) and/or attention deficit and hyperactivity disorder (ADHD). Front Psychiatry. 2019;10:673. doi:10.3389/fpsyt.2019.00673.
6. Avni E, Ben-Itzchak E, Zachor DA. The presence of comorbid ADHD and anxiety symptoms in autism spectrum disorder: clinical presentation and predictors. Front Psychiatry. 2018;9:717. doi:10.3389/fpsyt.2018.00717.
7. Gaugler T, Klei L, Sanders SJ, Bodea CA, Goldberg AP, Lee AB, Mahajan M, Manaa D, Pawitan Y, Reichert J, *et al.* Most genetic risk for autism resides with common variation. Nat. Genet. 2014;46:881–885. doi:10.1038/ng.3039.
8. Faraone SV, Larsson H. Genetics of attention deficit hyperactivity disorder. Mol Psychiatry. 2019;24:562–575. doi:10.1038/s41380-018-0070-0.
9. Satterstrom FK, Walters RK, Singh T, Wigdor EM, Lescai F, Demontis D, Kosmicki JA, Grove J, Stevens C, Bybjerg-Grauholm J, *et al.* Autism spectrum disorder and attention deficit hyperactivity disorder have a similar burden of rare protein-truncating variants. Nat. Neurosci. 2019;22(12):1961–1965. doi:10.1038/s41593-019-0527-8.
10. Buie T, Campbell DB, Fuchs GJ, Furuta GT, Levy J, VandeWater J, Whitaker AH, Atkins D, Bauman ML, Beaudet AL, *et al.* Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: A consensus report. Pediatrics. 2010;125(Supplement 1):S1–S18. doi:10.1542/peds.2009-1878C.
11. Vargason T, McGuinness DL, Gastrointestinal Symptoms HJ. Oral antibiotic use in children with autism spectrum disorder: retrospective analysis of a privately insured U.S. population. J Autism Dev Disord. 2019;49:647–659. doi:10.1007/s10803-018-3743-2.
12. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. Pediatrics Peds. 2014:2013–3995. doi:10.1542/peds.2013-3995.
13. Ming X, Chen N, Ray C, Brewer G, Kornitzer J, Steer RA. A gut feeling: a hypothesis of the role of the microbiome in attention-deficit/hyperactivity disorders. Child Neurology Open. 2018;5:2329048X18786799. doi:10.1177/2329048X18786799.
14. McKeown C, Hisle-Gorman E, Eide M, Gorman GH, Nylund CM. Association of constipation and fecal incontinence with attention-deficit/hyperactivity disorder. Pediatrics. 2013;132:e1210–5. doi:10.1542/peds.2013-1580.
15. Whiteley P, Haracopos D, Knivsberg A-M, Reichelt KL, Parlar S, Jacobsen J, Seim A, Pedersen L, Schondel M, Shattock P, *et al.* The scanbrit randomised, controlled, single-blind study of a gluten- and casein-free dietary intervention for children with autism spectrum disorders. Nutr. Neurosci. 2010;13:87–100. doi:10.1179/147683010X12611460763922.
16. Sonuga-Barke EJS, Brandeis D, Cortese S, Daley D, Ferrin M, Holtmann M, Stevenson J, Danckaerts M, van der Oord S, Döpfner M, *et al.* Nonpharmacological interventions for ADHD: systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. Am J Psychiatry. 2013;170:275–289. doi:10.1176/appi.ajp.2012.12070991.
17. Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen M-L, Bolte E, McTeague M, Sandler R, Wexler H, Marlowe E, *et al.* Gastrointestinal microflora studies in late-onset autism. Clin. Infect. Dis. 2002;35:S6–S16. doi:10.1086/341914.
18. Pärtty A, Kalliomäki M, Wacklin P, Salminen S, Isolauri E. A possible link between early probiotic intervention and the risk of neuropsychiatric disorders later in childhood: a randomized trial. Pediatr Res. 2015;77:823–828. doi:10.1038/pr.2015.51.
19. Sandler RH, Finegold SM, Bolte ER, Buchanan CP, Maxwell AP, Väisänen M-L, Nelson MN, Wexler HM. Short-term benefit from oral vancomycin treatment of regressive-onset autism. J. Child Neurol. 2000;15:429–435. doi:10.1177/088307380001500701.
20. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. Science [Internet] 2005;308:1635–1638.
21. Zhang X, Shen D, Fang Z, Jie Z, Qiu X, Zhang C, Chen Y, Ji L. Human gut microbiota changes reveal

the progression of glucose intolerance. PLoS One. 2013;8(8):e71108. doi:10.1371/journal.pone.0071108.
22. Turnbaugh P, Backhed F, Fulton L, Gordon J. Marked alterations in the distal gut microbiome linked to diet-induced obesity. Cell Host. 2008;3:213–223. doi:10.1016/j.chom.2008.02.015.
23. Maeda Y, Kurakawa T, Umemoto E, Motooka D, Ito Y, Gotoh K, Hirota K, Matsushita M, Furuta Y, Narazaki M, *et al*. Dysbiosis contributes to arthritis development via activation of autoreactive t cells in the intestine. Arthritis Rheumatol (Hoboken, N.J.). 2016;68:2646–2661. doi:10.1002/art.39783.
24. Emoto T, Yamashita T, Kobayashi T, Sasaki N, Hirota Y, Hayashi T, So A, Kasahara K, Yodoi K, Matsumoto T, *et al*. Characterization of gut microbiota profiles in coronary artery disease patients using data mining analysis of terminal restriction fragment length polymorphism: gut microbiota could be a diagnostic marker of coronary artery disease. Heart Vessels. 2017;32:39–46. doi:10.1007/s00380-016-0841-y.
25. Gevers D, Kugathasan S, Denson L, Vázquez-Baeza Y, Van Treuren W, Ren B, Schwager E, Knights D, Song S, Yassour M, *et al*. The treatment-naive microbiome in new-onset Crohn's disease. Cell Host Microbe. 2014;15(3):382–392. doi:10.1016/j.chom.2014.02.005.
26. Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S. Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. 2011;108:3047–3052. doi:10.1073/pnas.1010529108.
27. Slykerman RF, Coomarasamy C, Wickens K, Thompson JMD, Stanley TV, Barthow C, Kang J, Crane J, Mitchell EA. Exposure to antibiotics in the first 24 months of life and neurocognitive outcomes at 11 years of age. Psychopharmacology (Berl). 2019;236(5):1573–1582. doi:10.1007/s00213-019-05216-0.
28. Gorecki AM, Preskey L, Bakeberg MC, Kenna JE, Gildenhuys C, MacDougall G, Dunlop SA, Mastaglia FL, Akkari PA, Koengten F, *et al*. Altered gut microbiome in parkinson's disease and the influence of lipopolysaccharide in a human α-synuclein over-expressing mouse model. Front. Neurosci. 2019;13:839. doi:10.3389/fnins.2019.00839.
29. Cheung SG, Goldenthal AR, Uhlemann A-C, Mann JJ, Miller JM, Sublette ME. Systematic review of gut microbiota and major depression. Front Psychiatry. 2019;10:34. doi:10.3389/fpsyt.2019.00034.
30. Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y, Wang W, Tang W, Tan Z, Shi J, *et al*. Altered fecal microbiota composition in patients with major depressive disorder. Brain. Behav. Immun. 2015;48:186–194. doi:10.1016/j.bbi.2015.03.016.
31. Evans SJ, Bassis CM, Hein R, Assari S, Flowers SA, Kelly MB, Young VB, Ellingrod VE, McInnis MG. The gut microbiome composition associates with bipolar disorder and illness severity. Journal of Psychiatric Research. 2017;87:23–29. doi:10.1016/j.jpsychires.2016.12.007.
32. Sherwin E, Sandhu KV, Dinan TG, Cryan JF. May the force be with you: the light and dark sides of the microbiota-gut-brain axis in neuropsychiatry. CNS Drugs. 2016;30:1019–1041. doi:10.1007/s40263-016-0370-3.
33. Sgritta M, Dooling SW, Buffington SA, Momin EN, Francis MB, Britton RA, Costa-Mattioli M. Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. Neuron. 2019;101(246–259.e6). doi:10.1016/j.neuron.2018.11.018.
34. De Angelis M, Francavilla R, Piccolo M, De Giacomo A, Gobbetti M. Autism spectrum disorders and intestinal microbiota. Gut Microbes. 2015;6:207–213. doi:10.1080/19490976.2015.1035855.
35. de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, Iardino P, Carteni M, De Rosa M, Francavilla R, Riegler G, *et al*. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J. Pediatr. Gastroenterol. Nutr. 2010;51:418–424. doi:10.1097/MPG.0b013e3181dcc4a5.
36. Song Y, Liu C, Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. Appl Environ Microbiol. 2004;70:6459–6465. doi:10.1128/AEM.70.11.6459-6465.2004.
37. Parracho HMRT, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol. 2005;54:987–991. doi:10.1099/jmm.0.46101-0.
38. Sharon G, Cruz NJ, Kang D-W, Gandal MJ, Wang B, Kim Y-M, Zink EM, Casey CP, Taylor BC, Lane CJ, *et al*. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. Cell. 2019;177(1600–1618.e17). doi:10.1016/j.cell.2019.05.004.
39. Grimaldi R, Gibson GR, Vulevic J, Giallourou N, Castro-Mejía JL, Hansen LH, Leigh Gibson E, Nielsen DS, Costabile A. A prebiotic intervention study in children with autism spectrum disorders (ASDs). Microbiome. 2018;6(1):133. doi:10.1186/s40168-018-0523-3.
40. Shaaban SY, El Gendy YG, Mehanna NS, El-Senousy WM, El-Feki HSA, Saad K, El-Asheer OM. The role of probiotics in children with autism spectrum disorder: a prospective, open-label study. Nutr. Neurosci. 2018;21:676–681. doi:10.1080/1028415X.2017.1347746.
41. Ng QX, Loke W, Venkatanarayanan N, Lim DY, Soh AYS, Yeo WS. A systematic review of the role of prebiotics and probiotics in autism spectrum disorders. Medicina (Kaunas). 2019;55(5):129. doi:10.3390/medicina55050129.
42. Liu F, Li J, Wu F, Zheng H, Peng Q, Zhou H. Altered composition and function of intestinal microbiota in autism spectrum disorders: a systematic review. Transl Psychiatry. 2019;9. doi:10.1038/s41398-019-0389-6.
43. Leslie, Leslie DL, Kozma L, Martin A, Landeros A, Katsovich L, King RA, Leckman JF. Neuropsychiatric disorders associated with streptococcal infection: a

case-control study among privately insured children. J Am Acad Child Adolesc Psychiatry. 2008;47:1166–1172. doi:10.1097/CHI.0b013e3181825a3d.
44. Moher D, Liberati A, Tetzlaff J, Altman DG, Group PRISMA. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med. 2009;151:264–269. W64. doi:10.7326/0003-4819-151-4-200908180-00135.
45. Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D, *et al.* Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe. 2010;16(4):444–453. doi:10.1016/j.anaerobe.2010.06.008.
46. Gondalia SV, Palombo EA, Knowles SR, Cox SB, Meyer D, Austin DW. Molecular characterisation of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. Autism Res. 2012;5(6):419–427. doi:10.1002/aur.1253.
47. Rose DR, Yang H, Serena G, Sturgeon C, Ma B, Careaga M, Hughes HK, Angkustsiri K, Rose M, Hertz-Picciotto I, *et al.* Differential immune responses and microbiota profiles in children with autism spectrum disorders and co-morbid gastrointestinal symptoms. Brain. Behav. Immun. 2018;70:354–368. doi:10.1016/j.bbi.2018.03.025.
48. Zhang M, Ma W, Zhang J, He Y, Wang J. Analysis of gut microbiota profiles and microbe-disease associations in children with autism spectrum disorders in China. Sci Rep. 2018;8:13981. doi:10.1038/s41598-018-32219-2.
49. Coretti L, Paparo L, Riccio MP, Amato F, Cuomo M, Natale A, Borrelli L, Corrado G, De Caro C, Comegna M, *et al.* Gut microbiota features in young children with autism spectrum disorders. Front. Microbiol. 2018;9:3146. doi:10.3389/fmicb.2018.03146.
50. Li N, Yang J, Zhang J, Liang C, Wang Y, Chen B, Zhao C, Wang J, Zhang G, Zhao D, *et al.* Correlation of gut microbiome between ASD children and mothers and potential biomarkers for risk assessment. Genomics, Proteomics Bioinforma. 2019;17:26–38. doi:10.1016/j.gpb.2019.01.002.
51. Carissimi C, Laudadio I, Palone F, Fulci V, Cesi V, Cardona F, Alfonsi C, Cucchiara S, Isoldi S, Stronati L, *et al.* Functional analysis of gut microbiota and immunoinflammation in children with autism spectrum disorders. Dig. Liver Dis. 2019;51:1366–1374. doi:10.1016/j.dld.2019.06.006.
52. Liu S, Li E, Sun Z, Fu D, Duan G, Jiang M, Yu Y, Mei L, Yang P, Tang Y, *et al.* Altered gut microbiota and short chain fatty acids in Chinese children with autism spectrum disorder. Sci. Rep. 2019;9:287. doi:10.1038/s41598-018-36430-z.
53. Zhai Q, Cen S, Jiang J, Zhao J, Zhang H, Chen W. Disturbance of trace element and gut microbiota profiles as indicators of autism spectrum disorder: A pilot study of Chinese children. Environ. Res. 2019;171:501–509. doi:10.1016/j.envres.2019.01.060.
54. Ma B, Liang J, Dai M, Wang J, Luo J, Zhang Z, Jing J. Altered gut microbiota in chinese children with autism spectrum disorders. Front. Cell. Infect. Microbiol. 2019;9:40. doi:10.3389/fcimb.2019.00040.
55. Wang M, Wan J, Rong H, He F, Wang H, Zhou J, Cai C, Wang Y, Xu R, Yin Z, *et al.* Alterations in gut glutamate metabolism associated with changes in gut microbiota composition in children with autism spectrum disorder. mSystems. 2019;4(1):e00321–18. doi:10.1128/mSystems.00321-18.
56. Plaza-Díaz J, Gómez-Fernández A, Chueca N, Torre-Aguilar M, Gil Á, Perez-Navero J, Flores-Rojas K, Martín-Borreguero P, Solis-Urra P, Ruiz-Ojeda F, *et al.* Autism spectrum disorder (ASD) with and without mental regression is associated with changes in the fecal microbiota. Nutrients. 2019;11(2):337. doi:10.3390/nu11020337.
57. De Angelis M, Piccolo M, Vannini L, Siragusa S, De Giacomo A, Serrazzanetti DI, Cristofori F, Guerzoni ME, Gobbetti M, Francavilla R, *et al.* Fecal microbiota and metabolome of children with autism and pervasive developmental disorder not otherwise specified. PLoS One. 2013;8(10):e76993. doi:10.1371/journal.pone.0076993.
58. Kang D-W, Park JG, Ilhan ZE, Wallstrom G, LaBaer J, Adams JB, Krajmalnik-Brown R. Reduced incidence of prevotella and other fermenters in intestinal microflora of autistic children. PLoS One. 2013;8(7):e68322. doi:10.1371/journal.pone.0068322.
59. Son JS, Zheng LJ, Rowehl LM, Tian X, Zhang Y, Zhu W, Litcher-Kelly L, Gadow KD, Gathungu G, Robertson CE, *et al.* Comparison of fecal microbiota in children with autism spectrum disorders and neurotypical siblings in the simons simplex collection. PLoS One. 2015;10(10):1–19. doi:10.1371/journal.pone.0137725.
60. Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, Hayek J, Jousson O, Leoncini S, Renzi D, Calabrò A, *et al.* New evidences on the altered gut microbiota in autism spectrum disorders. Microbiome. 2017;5(1):24. doi:10.1186/s40168-017-0242-1.
61. Kang D-W, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, Khoruts A, Geis E, Maldonado J, McDonough-Means S, *et al.* Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome. 2017;5(1):10. doi:10.1186/s40168-016-0225-7.
62. Pulikkan J, Maji A, Dhakan DB, Saxena R, Mohan B, Anto MM, Agarwal N, Grace T, Sharma VK. Gut microbial dysbiosis in indian children with autism spectrum disorders. Microb. Ecol. 2018;76:1102–1114. doi:10.1007/s00248-018-1176-2.
63. Kang D-W, Ilhan ZE, Isern NG, Hoyt DW, Howsmon DP, Shaffer M, Lozupone CA, Hahn J,

Adams JB, Krajmalnik-Brown R, *et al.* Differences in fecal microbial metabolites and microbiota of children with autism spectrum disorders. Anaerobe. 2018;49:121–131. doi:10.1016/j.anaerobe.2017.12.007.
64. Berding K, Donovan SM. Diet can impact microbiota composition in children with autism spectrum disorder. Front Neurosci. 2018;12:515. doi:10.3389/fnins.2018.00515.
65. Aarts E, Ederveen THA, Naaijen J, Zwiers MP, Boekhorst J, Timmerman HM, Smeekens SP, Netea MG, Buitelaar JK, Franke B, *et al.* Gut microbiome in ADHD and its relation to neural reward anticipation. PLoS One. 2017;12(9):e0183509. doi:10.1371/journal.pone.0183509.
66. Prehn-Kristensen A, Zimmermann A, Tittmann L, Lieb W, Schreiber S, Baving L, Fischer A. Reduced microbiome alpha diversity in young patients with ADHD. PLoS One. 2018;13(7):e0200728. doi:10.1371/journal.pone.0200728.
67. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto J-M, *et al.* Enterotypes of the human gut microbiome. Nature. 2011;473(7346):174–180. doi:10.1038/nature09944.
68. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, *et al.* A core gut microbiome in obese and lean twins. Nature. 2009;457:480–484. doi:10.1038/nature07540.
69. Wu S, Rhee K-J, Albesiano E, Rabizadeh S, Wu X, Yen H-R, Huso DL, Brancati FL, Wick E, McAllister F, *et al.* A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat. Med. 2009;15:1016–1022. doi:10.1038/nm.2015.
70. Mancuso G, Midiri A, Biondo C, Beninati C, Gambuzza M, Macri D, Bellantoni A, Weintraub A, Espevik T, Teti G, *et al.* Bacteroides fragilis-derived lipopolysaccharide produces cell activation and lethal toxicity via toll-like receptor 4. Infect. Immun. 2005;73:5620–5627. doi:10.1128/IAI.73.9.5620-5627.2005.
71. Rao K, Erb-Downward JR, Walk ST, Micic D, Falkowski N, Santhosh K, Mogle JA, Ring C, Young VB, Huffnagle GB, *et al.* The systemic inflammatory response to clostridium difficile infection. PLoS One. 2014;9. doi:10.1371/journal.pone.0092578
72. Martínez I, Lattimer JM, Hubach KL, Case JA, Yang J, Weber CG, Louk JA, Rose DJ, Kyureghian G, Peterson DA, *et al.* Gut microbiome composition is linked to whole grain-induced immunological improvements. Isme J. 2013;7:269–280. doi:10.1038/ismej.2012.104.
73. Jiao N, Baker SS, Nugent CA, Tsompana M, Cai L, Wang Y, Buck MJ, Genco RJ, Baker RD, Zhu R, *et al.* Gut microbiome may contribute to insulin resistance and systemic inflammation in obese rodents: a meta-analysis. Physiol Genomics. 2018;50:244–254. doi:10.1152/physiolgenomics.00114.2017.
74. Kang D-W, Adams JB, Coleman DM, Pollard EL, Maldonado J, McDonough-Means S, Caporaso JG, Krajmalnik-Brown R. Long-term benefit of microbiota transfer therapy on autism symptoms and gut microbiota. Sci. Rep. 2019;9:5821. doi:10.1038/s41598-019-42183-0.
75. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen -Y-Y, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011;334:105–108. doi:10.1126/science.1208344.
76. Lacorte E, Gervasi G, Bacigalupo I, Vanacore N, Raucci U, Parisi P. A systematic review of the microbiome in children with neurodevelopmental disorders. Front. Neurol. 2019;10:727. doi:10.3389/fneur.2019.00727.
77. Vangay P, Johnson AJ, Ward TL, Al-Ghalith GA, Shields-Cutler RR, Hillmann BM, Lucas SK, Beura LK, Thompson EA, Till LM, *et al.* US immigration westernizes the human gut microbiome. Cell. 2018;175:962–972.e10. doi:10.1016/j.cell.2018.10.029.
78. Gomez-Arango LF, Barrett HL, Wilkinson SA, Callaway LK, McIntyre HD, Morrison M, Dekker Nitert M. Low dietary fiber intake increases Collinsella abundance in the gut microbiota of overweight and obese pregnant women. Gut Microbes. 2018;9(3):189–201. doi:10.1080/19490976.2017.1406584.
79. Miquel S, Martín R, Rossi O, Bermúdez-Humarán LG, Chatel JM, Sokol H, Thomas M, Wells JM, Langella P. Faecalibacterium prausnitzii and human intestinal health. Curr. Opin. Microbiol. 2013;16:255–261. doi:10.1016/j.mib.2013.06.003.
80. Shadnoush M, Hosseini RS, Khalilnezhad A, Navai L, Goudarzi H, Vaezjalali M. Effects of probiotics on gut microbiota in patients with inflammatory bowel disease : a double-blind, placebo-controlled clinical trial. Korean J. Gastroenterol. 2015;65:215–221. doi:10.4166/kjg.2015.65.4.215.
81. Cheng S, Han B, Ding M, Wen Y, Ma M, Zhang L, Qi X, Cheng B, Li P, Kafle OP. Identifying psychiatric disorder-associated gut microbiota using microbiota-related gene set enrichment analysis. Brief. Bioinform. 2019. doi:10.1093/bib/bbz034.
82. Zimmermann M, Zimmermann-Kogadeeva M, Wegmann R, Goodman AL. Mapping human microbiome drug metabolism by gut bacteria and their genes. Nature. 2019;570:462–467. doi:10.1038/s41586-019-1291-3.
83. Clemente JC, Pehrsson EC, Blaser MJ, Sandhu K, Gao Z, Wang B, Magris M, Hidalgo G, Contreras M, Noya-Alarcón Ó, *et al.* The microbiome of uncontacted Amerindians. Sci. Adv. 2015;1:e1500183–e1500183. doi:10.1126/sciadv.1500183.
84. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc. Natl. Acad. Sci.

U. S. A. 2010;107:14691–14696. doi:10.1073/pnas.1005963107.
85. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP. Human gut microbiome viewed across age and geography. Nature. 2012;486(7402):222–227. doi:10.1038/nature11053.
86. Zhang J, Guo Z, Xue Z, Sun Z, Zhang M, Wang L, Wang G, Wang F, Xu J, Cao H, *et al.* A phylo-functional core of gut microbiota in healthy young Chinese cohorts across lifestyles, geography and ethnicities. Isme J. 2015;9(9):1979–1990. doi:10.1038/ismej.2015.11.
87. Winglee K, Howard AG, Sha W, Gharaibeh RZ, Liu J, Jin D, Fodor AA, Gordon-Larsen P. Recent urbanization in China is correlated with a Westernized microbiome encoding increased virulence and antibiotic resistance genes. Microbiome. 2017;5(1):121. doi:10.1186/s40168-017-0338-7.
88. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505(7484):559–563. doi:10.1038/nature12820.
89. Emond A, Emmett P, Steer C, Golding J. Feeding symptoms, dietary patterns, and growth in young children with autism spectrum disorders. Pediatrics. 2010;126:e337–42. doi:10.1542/peds.2009-2391.
90. Siddiqi S, Urooj A, D'Souza MJ, Patterns D. Anthropometric measures of Indian Children with Autism spectrum disorder. J Autism Dev Disord. 2019;49:1586–1598. doi:10.1007/s10803-018-3850-0.
91. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. Gut. 2016;65(1):57–62. doi:10.1136/gutjnl-2015-309618.
92. Roesch LFW, Casella G, Simell O, Krischer J, Wasserfall CH, Schatz D, Atkinson MA, Neu J, Triplett EW. Influence of fecal sample storage on bacterial community diversity. Open Microbiol. J. 2009;3:40–46. doi:10.2174/1874285800903010040.
93. Albertsen M, Karst SM, Ziegler AS, Kirkegaard RH, Nielsen PH, Aziz RK. Back to basics - The influence of DNA extraction and primer choice on phylogenetic analysis of activated sludge communities. PLoS One. 2015;10(7):1–15. doi:10.1371/journal.pone.0132783.
94. Bundgaard-Nielsen C, Hagstrøm S, Sørensen S. Interpersonal variations in gut microbiota profiles supersedes the effects of differing fecal storage conditions. Sci Rep. 2018:1–9. doi:10.1038/s41598-018-35843-0.
95. Choo JM, Leong LE, Rogers GB. Sample storage conditions significantly influence faecal microbiome profiles. Sci Rep. 2015;5:16350. doi:10.1038/srep16350.
96. Rantakokko-Jalava K, Jalava J. Optimal DNA isolation method for detection of bacteria in clinical specimens by broad-range PCR. J Clin Microbiol. 2002;40:4211–4217. doi:10.1128/JCM.40.11.4211-4217.2002.
97. Park S-C, Won S. Evaluation of 16S rRNA databases for taxonomic assignments using mock community. Genomics Inform. 2018;16:e24. doi:10.5808/GI.2018.16.4.e24.