

Supplementary Materials for:

The Promising Role of Probiotics in Managing the Altered Gut in Autism Spectrum Disorders

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Table S1. A summary of the altered gut flora in individuals with autism spectrum disorder (ASD).

<i>Bacteria/Fungi</i>	Status in ASD cohorts (compared to neurotypical cohorts)	Significance	Reference
<i>Candida</i>	Higher	Secrete D- arabinitol Affects carbohydrate, and mineral absorption and Secretes toxin	[109, 110]
	Same		[101]
<i>Lactobacillus</i>	Lower	Normally maintain intestines' tight junctions.	[112]
	Higher		[103]
<i>Prevotella</i>	Lower	Normally degrades polysaccharides, important for community structure and vitamin B1 synthesis	[113]
<i>Veillonellaceae</i>	Lower	It ferments lactate	[108]
	Higher	The study used an omnivores diet	[118]
<i>Clostridium</i>	Higher	Directly correlated to GI problems	[103]
<i>Desulfovibrio</i>	Higher	Secrete hydrogen sulfide	[120]
<i>Streptococcus and Coprococcus</i>	decreased		[106]

Table S2. Summary of major animal studies of probiotic interventions, their mechanism of action and their outcomes related to ASD.

Name of Probiotic strains	Study type	Probiotic type and dose	Duration of intervention	Mechanism of action	Outcomes	Reference	year
Human-derived probiotic <i>Lactobacillus reuteri</i> strains (L. reuteri strains DSM17938, ATCC PTA4659, ATCC PTA 5289, and ATCC PTA 6475)	Newborn Sprague Dawley rat pups	10 ⁶ CFU.g body wt ⁻¹ .day ⁻¹ .	3 days	<ul style="list-style-type: none"> - Decreased level of IL13 and IFN- level in intestine by <i>L.reuteri</i> strain DSM 17938 - Decreased level in Lipopolysaccharides (LPS) induced TNF- and IL-1 - Decreased of intestinal histological damage produced by LPS - Affect Th1 type and Th2 type cytokines in rat intestine 	<ul style="list-style-type: none"> - Human-derived probiotic <i>Lactobacillus reuteri</i> strains differentially reduce intestinal inflammation - Recommendations: It is appropriate to evaluate different strains of the same probiotic carefully, because they may affect the host differently 	(165)	2010
<i>Bacteroides fragilis</i> NCTC 9343	Pregnant C57BL/6N mice	10 ¹⁰ CFU/ day	6 days	<ul style="list-style-type: none"> - Increased in expression of CLDNs 8 and 15 and restoration of colonic IL-6 levels linked to the correction of the gut barrier integrity and permeability in MIA offspring but also correction in tight junction and cytokine expression - Specific commensal intestinal microbiota restored in MIA offspring 	<ul style="list-style-type: none"> - It restored affected gut barrier evident by bringing serum metabolites, gut microbiota composition and behavioral symptoms back to normal in rat pups - Support a gut microbiome-brain connection in mouse model with ASD and the used probiotic may be considered as potential safe therapy for GI and particular behavior symptoms in ASD 	[98]	2013

				<ul style="list-style-type: none"> - Corrects ASD related behavioral and neurological abnormalities - Corrects level of production of serum metabolites involved in key metabolism pathways 		
Mixture of <i>bifidobacteria</i> and <i>Lactobacilli</i> strains (ProtexinR)	50 young male golden Syrian hamsters weighing between 60 and 70 g (7 weeks of age) in which autistic like behaviors were induced by clindamycin and propionic acid (PPA) administration	2×10^8 CFU/day	27 days	<ul style="list-style-type: none"> - Changes in the gut microbiota - ameliorates glutamate excitotoxicity through increasing depleted GABA and Mg^{2+} and decreasing the excitatory neurotransmitter, glutamate. 	<ul style="list-style-type: none"> - <i>Bifidobacteria</i> and <i>Lactobacilli</i> supplementation can be suggested as a strategy to ameliorate the glutamate excitotoxic effects of PPA and clindamycin. <p>→ Probiotic treatment reduces the autistic-like excitation/inhibition imbalance in juvenile hamsters</p>	[167] 2018
Probiotics Sachet Children's Formula (Biostime, Guangzhou, China): 1.9×10^8 cfu/g	C57BL/6J female mice	1.5675×10^7 cfu Bifidobacteria (<i>B. bifidum</i>)	21 days	<ul style="list-style-type: none"> - Increases in IL-6 and IL-17 levels in both maternal serum and fetal brain - Decreases in γ-aminobutyric acid levels in 	<p><i>Oral probiotics during pregnancy may be an effective means for decreasing the incidence of ASD in offspring</i></p>	[166] 2019

<p><i>Bifidobacteria</i> (<i>B. bifidum</i> and <i>B. infantis</i>) and 6.4×10^9 cfu/g <i>Lactobacillus helveticus</i>, in addition to fructooligosaccharides (FOS) and maltodextrin</p>	<p>and <i>B. infantis</i>) and 5.28×10^8 cfu <i>Lactobacillus helveticus</i>/24h</p>	<p>the prefrontal cortex of adult offspring</p> <ul style="list-style-type: none"> - Prevents maternal immune activation (MIA)-induced repetitive behaviors in adult offspring - Prevents MIA-induced anxiety-like behavior in adult offspring - Prevents MIA-induced social deficits in adult offspring - Prevents MIA-induced decreases in GABA levels in the PFC of adult offspring
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Table S3. Summary of major clinical studies of probiotic interventions, their mechanism of action and their outcomes related to ASD.

Name of Probiotic strains	Study type	Probiotic type and dose	Duration of intervention	Outcomes	Reference	year
General probiotics	Case report of 6-years-old boy with ASD	Not mentioned	8 weeks	- Improvement in school records and attitude against taking a variety of food - His behavior and situation reversed back when the supplementation with probiotics was stopped	[168]	2000
<i>Lactobacillus plantarum</i> WCFS1	Double blind placebo crossover trial in United Kingdom with 22 children with ASD aged between 3 and 16 years old (91 % males)	4.5×10^{10} CFUs daily	3 weeks	- Increased the number of <i>Lactobacilli/Enterococci</i> and decreases the <i>Clostridium coccoides</i> found in the stool samples of children with ASD as compared with placebo - Improvement of the anti-social behaviors, anxiety and communication problems - No major differences In GI symptoms	[119]	2010
<i>L. acidophilus</i>	Noncontrolled trial in Poland of 22 children with ASD aged between 4-10 years (90% males)	5×10^9 CFUs 2 times per day	8 weeks	- Decrease in the level of D-arabiinitol and D-/L-arabinotol in the urine of children with autism - Changes in ASD symptoms - Improvement in the ability to concentrate and to carry orders	[110]	2012
Delpro® (<i>Lactocillus acidophilus</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus delbruecki</i> , <i>Bifidobacteria longum</i> , <i>Bifidobacteria bifdum</i>) and Del Immune V® (<i>Lactobacillus rhamnosus</i> V lysate)	Noncontrolled trial in USA of 33 children with ASD aged between 3-16 years	10 billion CFUs total. 1 capsule, 3 times/d for 21 days	21 days	- Improvement in Autism Treatment Evaluation Checklist score (ATEC) for 88% of children - Improvement in GI symptoms, mainly reported a decrease in constipation and diarrhea,	[174]	2013
"Children Dophilus": Blend of: -3 strains of <i>Lactobacillus</i> (60%),	Noncontrolled trial in Slovakia of 29 Children: 10 ASD, 9 non-ASD siblings,	Not provided	3 times/day for 4 months	- Decreased levels of <i>Bacteroidetes/Firmicutes</i> ratio	[39]	2015

-2 strains of <i>Bifidumbacteria</i> (25%), -1 strain of <i>Streptococcus</i> (15%) (exact strain information not provided)	10 non-ASD controls aged between 2–9 y; non-ASD siblings = 5–17 y; non-ASD controls = 2–11 y			- Increase in the level of <i>Lactobacillus</i> in children with ASD as compared with controls - Increased level of - Increased in level of fecal TNF- α in children with ASD and their siblings compared with children without ASD, and there was a strong correlation between TNF- α concentrations and GI symptom		
<i>L. rhamnosus</i> GC (ATCC 53103)	Randomized trial, placebo controls in Finland of 75 infants for the 6 months of life and followed for 13 years starting at the age of 6 months	Not provided	The 6 first months of life	- At the age of 13 years, 6 out of 35 children who took placebo were diagnosed with ASD or attention-deficit hyperactivity disorder (ADHD), but none in probiotic group. - Probiotic supplementation early in life may reduce the risk of neuropsychiatric disorder development later in childhood by many mechanisms including the change in gut microbiota composition.	[170]	1015
VSL#3 (VSL Pharmaceuticals, Ft Lauderdale, FL, USA) : 3 \times 10 ¹¹ CFU/g of bifidobacteria (<i>B. longum</i> , <i>B. infantis</i> and <i>B. breve</i>), 8 \times 10 ¹⁰ CFU /g of lactobacilli (<i>L. acidophilus</i> , <i>L. casei</i> , <i>L. delbrueckii</i> subsp. <i>L. bulgaricus</i> and <i>L. plantarum</i>) and 20 \times 10 ¹⁰ CFU /g of <i>Streptococcus salivarius</i> subsp. <i>thermophilus</i> .	Case report of 12-years old boy with ASD and severe cognitive disability	Not provided	4 weeks followed by 4 months follow up	- Reduction in the severity of abdominal symptoms - Reduction in neurobehavioral and gastrointestinal symptoms	[169]	2016
Vivomixx probiotic: <i>Streptococcus thermophilus</i> , <i>Bifidobacterium breve</i> , <i>Bifidobacterium longum</i> ,	Randomized, placebo-controlled trial of a group of 100 preschoolers with	Dietary Supplement: Vivomixx®	5 months	- Changes in severity level of ASD and GI symptomatology - Changes in Behavioral Profiles - Changes in Parental Stress	[59]	2016

<p><i>Bifidobacterium infantis</i>, <i>Lactobacillus acidophilus</i>, <i>Lactobacillus plantarum</i>, <i>Lactobacillus paracasei</i>, <i>Lactobacillus delbrueckii subsp. bulgaricus</i></p>	<p>ASD aged between 18-72 months in Italy. Blind randomized 1:1 to regular diet with probiotic or with placebo for 5 months</p>	<p>Two packets (900 billions of bacteria) per os (P.O.) daily x 1 month and one packet (450 billions of bacteria) P.O. daily x 5 months Dietary Supplement: PlaceboTwo packets (4,4 grams of maltose and silicon dioxide x 2) P.O. daily x 1 month and one packet (4,4 grams of maltose and silicon dioxide) P.O. daily x 5 months</p>	<p>- Changes in Electroencephalogram obtained parameters - Changes in levels of serum Lipopolysaccharide, leptin, serum resistin, TNF , IL-6, PAI-1, fecal calprotectin</p>	<p>[173]</p>	<p>2018</p>	
<p>3 strains: <i>Lactobacillus acidophilus</i>, <i>Lactobacillus rhamnosus</i>, <i>Bifidobacteria longum</i></p>	<p>Prospective, open label noncontrolled trial in Egypt of 60 Children: 30 ASD and 30 age-/sex-matched controls (relatives) aged between 5-9 years old</p>	<p>5 g of powder/day (each gram contained 100x 10⁶ CFUs of each strain)</p>	<p>1 time/day for 3 months</p>	<p>- Increase in <i>Bifidobacterial</i> and <i>Lactobacilli</i> levels - Improvement in the severity of autism assets by the decrease in ATEC score - Improvements in GI problems the probiotic has beneficial effect on both behavioral and GI manifestations of ASD</p>	<p>[173]</p>	<p>2018</p>

Could be recommended for children with ASD as an adjuvant therapy

<p><i>Lactobacillus rhamnosus</i> HN001 Or <i>Bifidobacteria animalis</i> subsp. <i>lactis</i> HN019</p>	<p>Two centers, randomized, double blind, placebo-controlled study of 342 children followed from birth to 11 years in New Zealand</p>	<p>Not provided</p>	<p>Mothers given probiotics from 35 weeks pregnant until 6 months. Children receive the treatment from birth to 2 years</p>	<p>- HN001 and HN019 given in early life were not associated with neurocognitive outcomes at 11 years of age. - Recommendations: the study cannot exclude that other probiotics may have beneficial effect.</p>	<p>[59]</p>	<p>2018</p>
<p><i>Bifidobacterium infantis</i> in combination with a prebiotic bovine colostrum product (BCP)</p>	<p>A double-blind, crossover, randomized clinical trial with children ages 2–11 with ASD and GI comorbidities</p>	<p>A dose of 4 x 10⁹ CFU twice daily of probiotics and 0.15 g/lb body weight per day for the prebiotics</p>	<p>12-week study included 5 weeks of probiotic-prebiotic supplementation, followed by a two-week washout period, and 5 weeks of prebiotic only supplementation</p>	<p>- Reduction in the frequency of certain GI symptoms - Reduced occurrence of particular aberrant behaviors - Reduction in IL-13 and TNF-α production in some participants Although there is some improvements in ASD children's, further research into the efficacy of these treatments should be done.</p>	<p>[172]</p>	<p>2019</p>

<p>VISBIOME probiotic: <i>Streptococcus thermophilus</i>, <i>Bifidobacterium breve</i>, <i>Bifidobacterium longum</i>, <i>Bifidobacterium infantis</i>, <i>Lactobacillus acidophilus</i>, <i>Lactobacillus plantarum</i>, <i>Lactobacillus paracasei</i>, <i>Lactobacillus delbrueckii subsp. bulgaricus</i></p>	<p>Randomized crossover trial with 13 children</p>	<p>Not mentioned</p>	<p>19 weeks</p>	<p>- The VISBIOME formulation was safe and suggested a health benefit in children with ASD and GI symptoms who retained <i>Lactobacillus</i></p>	<p>[174]</p>	<p>2020</p>
<p><i>L. reuteri</i> (BioGaia)</p>	<p>Two staged, randomized, double blind placebo controlled, parallel group study of 60 patients with ASD aged between 3-25 years old in USA.</p>	<p>Dietary Supplement: oral probiotics 200 million cfu per day for 24 weeks Dietary Supplement: oral placebo 2 pills per day for 24 weeks</p>	<p>24 weeks</p>	<p>- Social communication and behavior test - Neuroinflammation and Oxytocin levels - structural and functional MRI - 16s metagenomic sequencing of the microbiome - Vital indices measurements - Assessment of GI function and gut microbiome/SCAFs</p>	<p>[171]</p>	<p>2020</p>

Table S4. Summary of ongoing human studies of probiotic interventions to ASD.

NCT No.	Trial name	Interventions	Probiotic type. dose and	Intervention Model	Outcomes measures	Estimated enrollment	Sponsors	Status
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		duration of intervention							
NCT03514784	Combination Probiotic: BB-12 With LGG (Different Doses) in Treating Children With Autism Spectrum Disorder	Drug: BB-12 with LGG (Higher Dose) Drug: Placebo Drug: BB-12 with LGG (Lower Dose)	Drug: BB-12 with LGG (Higher Dose: 10 billion CFUs) Drug: Placebo Maltodextrin Drug: BB-12 with LGG (Lower Dose; 1 billion CFUs) Over 56-day period and a 28-day observation period	Blinded randomized controlled study	<ul style="list-style-type: none"> - Effects of BB-12+LGG at different doses on GI symptoms - Effects of BB-12+LGG at different doses on gut inflammation (S1009A) - Effects of BB-12+LGG at different doses on gut inflammation (Fecal Calprotectin, plasma Zonulin and IL-8) - Using 16S rDNA analysis, this will determine microbial alpha and beta diversity and distribution of the major taxa before and after probiotic treatment - Effects of BB-12+LGG at different doses on metabolites to determine levels of metabolic markers which are reported to be abnormal in autism: fecal amino acids, ammonia, short chain fatty acids (SCFA), and phenols. 	70 healthy children with confirmed ASD status	UTHealth Houston, Texas, United States	Recruiting	

NCT02903030	Efficacy of Vivomixx on Behavior and Gut Function in Autism Spectrum Disorder	Dietary Supplement: Vivomixx Dietary Supplement: Placebo	Probiotic: multi strain probiotic Vivomixx containing 450 billion lyophilized bacterial cells per sachet belonging to 8 probiotic strains. Placebo: 4.4 grams of maltose and silicon dioxide per sachet Duration: 3 months supplementation	Randomized controlled cross-over design with 4-week washout	<ul style="list-style-type: none"> - A change in the Autism Treatment Evaluation Checklist (ATEC) total score compared to baseline. - A change in the frequency of gastrointestinal symptoms compared to baseline, as measured by the Gastrointestinal History (GIH) questionnaire. - A change in the Aberrant Behavior Checklist (ABC) total score compared to baseline. - A change in the Autism Parenting Stress Index (APSI) score compared to baseline. 	82 children with Autism Spectrum Disorders and co-morbid gastrointestinal symptoms	Ohio State University Wexner Medical Center Columbus, Ohio, United States	Recruiting
NCT02903030	Probiotics for Quality of Life in Autism Spectrum Disorders	Drug: BB-12 with LGG Drug: Placebo	Drug: combination probiotic BB-12 with LGG orally (LGG dose: 10 ⁹ c.f.u.'s; BB-12: 10 ⁹) for a total of 56 doses Pplacebo (maltodextrin) at 2:1 ratio. Duration: 84 days	Randomized	<ul style="list-style-type: none"> - Children with ASD & GI symptoms differ in microbiome composition and function from neurotypical children with GI symptoms - Visbiome Extra Strength, improves GI and pain symptoms, correlating with altered gut microbiome 	13	Ohio State University Wexner Medical Center Columbus, Ohio, United States	Completed Has Results

					composition and related metabolites			
					- Change in The Aberrant Behavior			
					- Change in Social Responsiveness Scale			
NCT02674984	Road to Discovery for Combination Probiotic BB-12 With LGG in Treating Autism Spectrum Disorder	Drug: BB-12 with LGG Drug: Placebo	<p>Drug: Visbiome Extra Strength It is a mix of 8 strains of beneficial bacteria that should improve gut flora, improving GI function and hopefully anxiety.</p> <p>Drug: Maltose (placebo) Maltose with a trace amount of silicon dioxide Other Name: Maltose with silicon dioxide Duration: 8 weeks per condition with 3 weeks washout between</p>	Randomized	<p>- Effect of combination probiotic BB-12+LGG on adverse events assessed by case report form (survey) that are related to BB-12 with LGG (health-promoting bacteria) or placebo treatment.</p> <p>- Effect of combination probiotic BB-12+LGG on irritability and maladaptive behaviors with Aberrant Behavior Checklist (ABC)</p> <p>- Effect of combination probiotic BB-12+LGG on irritability and maladaptive behaviors measured with the Social Responsiveness Scale-2</p>	29 healthy children with autism spectrum disorder aged 4-15 years	University of Texas Health Science Center at Houston Houston, Texas, United States	Completed

NCT04293783	Randomized Double-blind Clinical Trial With <i>L.Reuteri</i> Supplementation in Children With Autism Spectrum Disorder	Dietary Supplement: <i>L.Reuteri</i> Dietary Supplement: Placebo	Dietary Supplement: <i>L.Reuteri</i> daily supplementation with two tablets for six months Other Name: Gastrus Dietary Supplement: <i>L.Reuteri</i> daily supplementation with two tablets for six months Other Name: Gastrus	Double- blind randomized, parallel- group, placebo- controlled study	- Changes in GI and ASD symptoms - Changes in Adaptive Functioning - Changes in Behavioral Profiles and parental stress Changes in Metabolomic Profile such as neurotransmitter metabolism, gastrointestinal alterations or dysbiosis, and mitochondrial dysfunction - The effect of probiotic administration on the metabolome will be investigated. - Changes in inflammatory profile (zonulin, IL-17 profile)	80 children With Autism Spectrum Disorder	University of Bari Bari, BA, Italy University of Rome Tor Vergata Rome, RM, Italy	Recruiting
NCT03982290	Psychophysiological Effects of <i>Lactobacillus</i> <i>Plantarum</i> PS128 in Preschool Children With Autism Spectrum Disorder	Probiotic, Lactobacillus <i>plantarum</i> PS128 Placebo: microcrystalline cellulose	Probiotic, <i>Lactobacillus</i> <i>plantarum</i> PS128 PS128 capsules, 2 capsules per day Other Name: PS128 Placebo, microcrystalline cellulose	Double- blinded, randomized, placebo- controlled	- Changes from baseline of anxiety subscale in Children's Behavior Checklist (CBCL)/Achenbach System of Empirically Based Assessment (ASEBA) at week 8 and week 16 - Changes from hyperactivity subscale of	250 Preschool Children with Autism Spectrum Disorder	Department of Pediatric Neurology, MacKay Children's Hospital Taipei, Taiwan	Recruiting

			Placebo capsules, 2 capsules per day Duration: 16 weeks		Attention-Deficit/Hyperactivity Disorder Test (ADHDT) at week 8 and week 16 - Gut and oral microbiota analysis - Changes in constipation, diarrhea, stool consistency, stool smell, flatulence, and abdominal pain - Changes in Communicative and interactive assessment			
NCT03858816	Efficacy of Multiple Strain Probiotics Reduces the Neurobehavioral Disorder in Premature Very Low Birth Weight Infants	Mixture probiotics Other: Placebo	The mixture probiotics capsule: contain 1×10^9 CFU/1 capsule of mixture probiotics, taking 1 probiotic capsule for up to 4 months after birth. The placebo contains the same excipient ingredients but without the live bacteria: 1 placebo capsule	Double blind, randomized, controlled trial	- Incidence rate of death or ADHD and ASD - Incidence rate of NEC \geq stage 2, sepsis, severe (grade 3-4) IVH, BPD, liver function and adverse effects or intolerance and neurodevelopment impairment.	320 Preterm infants \geq 23 weeks and \leq 32 weeks gestational age and birth weight below 1500 gm and who survive to NICU	Department of Pediatrics, Children Hospital, China Medical University Taichung, Taiwan	Recruiting

			for up to 4 months after birth					
NCT02086110	Effect of Milk Oligosaccharides and Bifidobacteria on the Intestinal Microbiota of Children with Autism	Milk Oligosaccharides (prebiotic) and Bifidobacteria (probiotic)	oligosaccharides in two divided doses per day orally Prebiotic: bovine colostrum, bovine oligosaccharides administered orally twice per day for a daily total of 0.3 g per pound of body weight for the first five weeks, followed by a two-week break with no treatment, and then will receive the synbiotic Probiotic: <i>Bifidobacterium infantis</i> SC268 (10 billion CFU) twice a day plus 0.3 g per pound body weight of bovine milk for	Randomized	- Stool Microbiota Composition Change During Prebiotic Only Treatment - Stool Microbiota Composition Change During Synbiotic Treatment - Serum Immune Profile Change During Prebiotic Only Treatment	11 children with Autism	UC Davis MIND Institute Sacramento, California, United States	Completed Has Results

the next five
weeks
Duration: 8
weeks
