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Effect of probiotic supplementation on cognitive function in children and adolescents: a systematic review of randomised trials

D. Rianda[1], R. Agustina[1,2*], E.A. Setiawan[1] and N.R.M. Manikam[1]

[1]Department of Nutrition, Faculty of Medicine, Universitas Indonesia – Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia; [2]Human Nutrition Research Center, Indonesian Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia; dr.rinaagustina@gmail.com; r.agustina@ui.ac.id

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REVIEW ARTICLE

Abstract

Available reviews have shown potential effects of probiotics on neurobehavioral outcomes through 'gut-brain axis' mechanism in adults. However, reviews on cognitive function in children and adolescents are lacking. Therefore, we conducted a systematic review of randomised controlled trials (RCTs) of the effect of probiotic supplementation on cognitive function in children and adolescents. A search of four databases (Cochrane Central Register of Controlled Trials, PsycARTICLES, Scopus, PubMed) was conducted to identify RCTs published from January 1990 to December 2018. Seven studies met the inclusion criteria and their cognitive outcomes were analysed. Only one study found a positive result with *Lactobacillus rhamnosus* GG (LGG) 1×10^{10} cfu supplementation with outcomes on attention deficit hyperactivity disorder (ADHD) or Asperger syndrome (AS) manifestations as diagnosed using the International Classification of Diseases-10 criteria. The supplementations were administered to Finnish mothers for 4 weeks before delivery and continuously given for 6 months after delivery if they breastfed, or to the children. ADHD or AS was diagnosed at the age of 13 years in 17.1% children in the placebo and none in the probiotic group (P=0.008). This study found significant differences in species composition and number of cells belonging to the genus *Bifidobacterium* between healthy children and children who later developed ADHD or AS at different time points. Six remaining studies with varying strains, durations of intervention, start-time of administration, and outcomes demonstrated no difference in cognition after probiotic supplementation. Metagenomic analyses on gut microbiota composition were not performed in any of these studies. In conclusion, the favourable effect of probiotic supplementation on cognitive function in children and adolescents was observed in one study with LGG supplementation by a risk reduction of developing ADHD or AS (i.e. autism). More long-term and follow-up trials using probiotics identifying the effect on cognition are warranted before routine use.

Keywords: adolescents, autism, children, probiotics

1. Introduction

Cognition is one of the most important aspects of child development. It implies the ability to think, learn, and remember, thus forming the basis for an individual's capacity for perception, reasoning, creativity, problem-solving, and possibly intuition (Antony *et al.*, 2017). Cognition encompasses many processes such as attention, processing speed, and memory (Hughes and Bryan, 2003). Notably, childhood period provides a sensitive phase of brain development, and is associated with a long-term impact on economic outcomes, such as earnings, occupational status, and national economic performance (Bornstein, 1989; Colombo, 1982; Hanushek and Woessmann, 2008; Kerckhoff *et al.*, 2001; Vertes and Bullmore, 2015). As the population under 25 years old (including children and adolescents) made up 42% of the world population in 2017 (World Bank, 2017), optimising brain development and cognitive function in this population will contribute to a significant number of world population with better quality

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of human resources. However, the complex interaction between biomedical and socioenvironmental factors affecting cognition, particularly during childhood, signifies the importance of an innovative approach and strategy to optimise cognitive development (Prado *et al.*, 2017). Therefore, focusing on cognitive outcome is crucial for improving the quality of early childhood development, health, and well-being in children and adolescents, and has become part of global strategy to accelerate the achievement of Sustainable Development Goals (Every Women Every Child, 2015).

Among biomedical factors (e.g. fatty acids, iron, iodine), there is a growing interest in the role of probiotics in optimising cognitive development. According to the World Health Organization, probiotics are live microorganisms which, when administered in adequate amounts, confer health benefits to the host (FAO/WHO, 2002). Based on the theory of 'gut-brain axis', it is hypothesised that the modulation of gut microbiota might affect brain function through immunological, biochemical, and neuroendocrine mechanisms (Galland, 2014). E.g. probiotic supplementation may provide beneficial bacteria which can increase the production of short chain fatty acids (SCFAs) (Louis and Flint, 2017; Rivière *et al.*, 2016). SCFAs have a range of systemic effects, including anti-inflammatory properties, to support blood-brain barrier integrity, and stimulation of brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) production for neuronal and glial development, neuroprotection, and modulation of synaptic interactions (Galvez-Contreras *et al.*, 2016; Stilling *et al.*, 2016; Wu *et al.*, 2008). A randomised controlled trial (RCT) by Akbari *et al.* (2016) showed a significant improvement in the Mini-Mental State Examination (MMSE) score in patients with Alzheimer Disease after supplementation of multispecies potential probiotic milk containing *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, and *Lactobacillus fermentum* (2×10^9 cfu/g each) for 12 weeks (Akbari *et al.*, 2016). Similarly, another RCT by Xia *et al.* (2018) found a significant improvement in digit symbol test and number connection test results after supplementation of a probiotic mixture containing *Clostridium butyricum* CGMCC0313-1 1×10^7 cfu/g combined with *Bifidobacterium infantis* CGMCC0313-2 1×10^6 cfu/g in patients with hepatitis B virus (HBV)-induced cirrhosis (Xia *et al.*, 2018).

While several studies have reviewed the effect of probiotics on neurobehavioral outcomes in adults, reviews on cognitive function in children and adolescents are lacking. A review by Wang *et al.* (2016) showed the efficacy of probiotics in improving psychiatric disorder-related behaviours in animals and humans (Wang *et al.*, 2016). Wallace and Milev (2017) have summarised the effect of probiotics on mood, stress, anxiety, and cognition in populations between 18-75 years old, and the majority of included studies found compelling results (Wallace and Milev, 2017). Recently, Upadhyay *et al.* (in press) published a meta-analysis synthesising the effect of prebiotic and probiotic supplementation on various neurodevelopmental outcomes including cognitive function in preterm, very low birth weight infants. Nonetheless, their study did not find any difference between prebiotic/probiotic-treated and untreated control groups in any of the neurodevelopmental outcomes.

This review aimed to systematically evaluate the effect of probiotic interventions on cognitive function in children and adolescents. Interventions using synbiotics or probiotics alone were included in this review. Assessment of cognitive outcomes included behavioural examinations [e.g. Bayley Scales for Infants Development (BSID), Wechsler Intelligence Scale for Children (WISC)] or diagnoses of conditions related to cognitive impairment [e.g. attention deficit hyperactivity disorder (ADHD)]. To broaden our understanding, we also intentionally looked for cognitive impairment in specific conditions such as autism.

2. Materials and methods

Study selection criteria and search strategy

Two authors (D.R., E.A.S) separately searched for publications using Cochrane Central Register of Controlled Trials (CENTRAL), PsycARTICLES, Scopus, and PubMed within a restricted time period between January 1, 1990, until December 31, 2018. To be included, the study had to be a randomised controlled trial of probiotic or synbiotic supplementation in children or adolescents (<18 years old), had cognitive function assessed at any point of time after supplementation, and had to be a human trial. Publications were restricted to English only and unpublished studies were not included in this review. The search terms used were 'probiotic*', 'bifidobacter*', 'lactobacillus', 'cognit*', 'processing speed', 'attenti*', 'memory', 'executive function', 'neurodevelopment*', 'bayley', 'intelligence quotient', 'attention deficit hyperactivity disorder', and 'autis*'. These terms were adapted for each bibliographic database in combination with database-specific filters for clinical trials in human.

After initial title and abstract screening, two authors (D.R., E.A.S.) independently reviewed the full-text version of potentially relevant studies for eligibility. To assess the quality of included studies, we contacted the corresponding authors for studies with undisclosed data or requiring clarification. Any discrepancy was discussed with the senior authors (R.A., N.R.M.M.). The protocol of the systematic review was registered into the International Prospective Register of Systematic Reviews (registration number CRD42018115313).

Data extraction and quality control of selected studies

Data of eligible studies were extracted into tabular form. Data extraction included information on the first author, year of publication, general characteristics of the study population, number of subjects for each group, intervention and control product, duration of intervention, the cognitive outcome assessed, the relevant measurement used, and results on cognitive outcome. Two authors (D.R., E.A.S.) independently assessed the quality of included studies using the Cochrane 'Risk of Bias Assessment Tool' comprising random sequence generation, allocation concealment, selective reporting, blinding of participants, blinding of personnel, blinding of outcome assessment team, attrition bias, and identification of other potential biases (Higgins and Green, 2011). The Cochrane Risk of Bias summary is provided in Supplementary Table S1. To report relevant items for publication, we used the PRISMA checklist for systematic reviews.

3. Results

A flow diagram of the article selection process is presented in Figure 1. A total of 328 articles were collected using online databases. After excluding 67 duplicates, we screened the titles and abstracts of 261 studies. We excluded 254 studies for various reasons (e.g. observational studies, no cognition as an outcome, not in children or adolescents). Finally, we assessed seven studies for eligibility (Akar *et al.*, 2017; Chou *et al.*, 2010; Firmansyah *et al.*, 2011; Jacobs *et al.*, 2017; Partty *et al.*, 2015; Sari *et al.*, 2012; Slykerman *et al.*, 2018). Based on the quality assessment, all studies had low risk of biases (Supplementary Table S1). All remaining studies were included in qualitative synthesis. Details of the included studies are provided in Table 1.

Almost all studies were conducted in upper-middle-income and high-income countries (Akar *et al.*, 2017; Chou *et al.*, 2010; Jacobs *et al.*, 2017; Partty *et al.*, 2015; Sari *et al.*, 2012; Slykerman *et al.*, 2018) with only one study performed in Indonesia, a lower-middle-income country (Firmansyah *et al.*, 2011). In four studies, probiotics were administered to preterm, very low birth weight infants until discharge (Akar *et al.*, 2017; Chou *et al.*, 2010; Jacobs *et al.*, 2017; Sari *et al.*, 2012). Of these 4 studies, information on the length of hospitalisation in 2 studies (Akar *et al.*, 2017; Jacobs *et al.*, 2017) were based on the other articles published elsewhere (Jacobs *et al.*, 2013; Oncel *et al.*, 2014) from the same trial that were cited by the authors. For the study by Sari *et al.* (2012), the information on days of intervention duration was not provided in the article nor the other article elsewhere (Sari *et al.*, 2011, 2012). Furthermore, probiotic administrations in studies by Partty *et al.* (2015) and Slykerman *et al.* (2018) were started from 35 weeks gestation or 4 weeks before expected delivery and continuously given to mothers for 6 months if they breastfed, or to their children (Partty *et al.*, 2015; Slykerman *et al.*, 2018). For probiotic formulation, about half of the

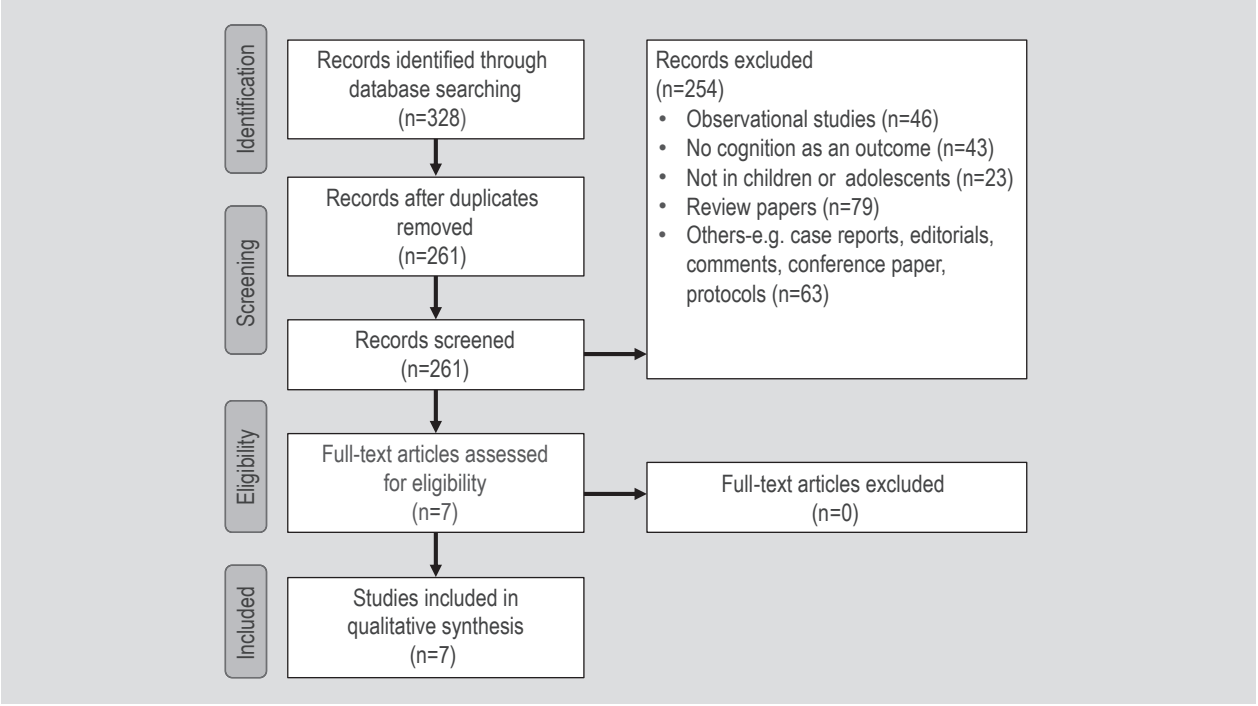

Figure 1. Flow diagram depicting the article selection process for a systematic review of the effect of probiotic supplementation on cognitive function in children and adolescents.

Table 1. Details of the studies included in the qualitative analysis of the effect of probiotic supplementation on cognitive function in children and adolescents.[1]

Reference	Population	Nr. of participants (PF; PL)	Product		Duration of intervention (days)	Cognitive outcomes assessed	Results
			Intervention	Control			
Chou *et al.* (2010)	Preterm with very low birth weight infants	301 (PF=153; PL=148)	PF: Infloran® capsule (multi-strain probiotic containing *Lactobacillus acidophilus* obtained from the ATCC in 1973 1×10^9 cfu, and *Bifidobacterium infantis* obtained from the ATCC in 1973 – Swiss Serum and Vaccine Institute, Berne, Switzerland 1×10^9 cfu in powder form) 125 mg/kg per dose, twice daily with breastmilk	Breast milk without the addition of probiotic	On day 7 after birth until discharge (46.3±25.9 days in PF group vs 47.4±24.1 days in PL group)	MDI and cognitive delay[2] at the age of 3 years corrected age (BSID-II)	No effect on MDI scores and proportion of cognitive delay at follow up
Firmansyah *et al.* (2011)	Healthy children aged 12 months old	153 (PF=76; PL=77)	PF: Milk containing *Bifidobacterium longum* BL99 (ATCC: BAA 999) 1×10^7 cfu, *Lactobacillus rhamnosus* LPR (CGMCC 1.3724) 2×10^7 cfu, prebiotics inulin (1.02 g) and FOS (2.38 g), and LCPUFA per 100 g (14.4 g/100 ml of reconstituted milk, given 2×200 ml per day)	Control milk	360	Cognitive and language scores (Bayley-III)	No effect on cognitive scores and language scores over time
Sari *et al.* (2012)	Preterm with very low birth weight infants	174 (PF=86; PL=88)	PF: Potential probiotic of *L. sporogenes* [*Bacillus coagulans* according to Bergey's Manual (Breed *et al.*, 1957)] (DMG ITALIA SRL, Rome, Italy) 3.5×10^8 cfu in a suspension of freeze-dried powder, once daily with breastmilk or formula	Fed with breastmilk or formula without the addition of *B. coagulans*	Started from the first feed until discharge	MDI and cognitive delay[2] at the age of 18 to 22 months' corrected age (BSID-II)	No effect on MDI scores and proportion of cognitive delay at follow up
Partty *et al.* (2015)	Infants who had at least one family member with an allergic disease	75 (PF=40; PL=35)	PF: Capsule containing *L. rhamnosus* GG (ATCC 53103) 1×10^{10} cfu	Capsule containing micro-crystalline cellulose	208 (given to mothers before expected delivery for 4 weeks, given either to children or continuously to the mothers if they breastfed their children for 6 mo)	Incidence of ADHD or AS at the age of 13 years old (diagnosed by an experienced child psychiatrist or neurologist using ICD-10)	Significant effect on the incidence of ADHD/AS; PF = 0/40 (0%); PL = 6/35 (17.1%); (P=0.008)
Akar *et al.* (2017)	Preterm with very low birth weight infants	249 (PF=124; PL=125)	PF: *Lactobacillus reuteri* DSM 17938 (Biogaia AB, Stockholm, Sweden) 1×10^8 cfu (5 drops), once daily with breastmilk or formula	Fed without the addition of *L. reuteri*	Started from the first feed until discharge [38 (10-131) days in PF group vs 46 (10-180) days in PL group] (Oncel *et al.*, 2014)	MDI and cognitive delay[2] at the age of 18 to 24 months' corrected age (BSID-II)	No effect on MDI scores and proportion of cognitive delay at follow up

Table 1. Continued.

Reference	Population	Nr. of participants (PF; PL)	Product		Duration of intervention (days)	Cognitive outcomes assessed	Results
			Intervention	Control			
Jacobs *et al.* (2017)	Preterm with very low birth weight infants	664 (PF=337; PL=327)	PF: *B. infantis* (BB-02 96579) 3×10^8 cfu, *Streptococcus thermophilus* (TH-4 15957) 3.5×10^8 cfu, *Bifidobacterium lactis* (BB-12 15954) 3.5×10^8 cfu in maltodextrin base powder (ABC Dophilus Probiotic Powder for Infants, Solgar, USA)	Malto-dextrin powder	Started from 72 h after birth until discharge [71 (54-92) days in PF group vs 74 (58-93) days in PL group] (Jacobs *et al.*, 2013) or term corrected age	Cognitive impairment[3], cognitive scores, and language scores at the age of 2-5 years corrected age (Bayley-III or WPPSI-III)	No effect on cognitive impairment, cognitive scores, language scores, and FSIQ at follow up
Slykerman *et al.* (2018)	Infants at risk of developing allergic disease	342 (PF1=109; PF2=118; PL=115)	PF1: Capsule containing *L. rhamnosus* HN001 6×10^9 cfu in powder form; PF2: Capsule containing *B. lactis* HN019 9×10^9 cfu in powder form	Placebo capsule containing dextran, salt, and yeast extract	751 (given to mothers from 35 weeks gestation until six months if breastfeeding, given to children from birth until two years)	Intelligence, executive function, attention at the age of 11 years old (WISC-IV, Conners 3™, Conners CPT 3™, CANTAB)	No effect on FSIQ, Conners 3™ parent T-scores, Conners CPT 3™ T-scores, and CANTAB at follow up

[1] ADHD = Attention Deficit Hyperactivity Disorder; AS = Asperger syndrome; ATCC = American Type Culture Collection; Bayley-III = Bayley Scale of Infant and Toddler Development-III; BSID-II = Bayley Scale of Infant and Toddler Development-II; CANTAB = Cambridge Neuropsychological Test Automated Battery; Conners 3™ = Conners 3rd edition; Conners CPT 3™ = The Conners Continous Performance Test 3rd edition™; FOS = fructooligosaccharides; FSIQ = Full Scale Intelligence Quotient; ICD-10 = International Classification of Diseases 10th revision; MDI = Mental Development Index; PF = probiotic formulation; PL = placebo; WISC-IV = Wechsler Intelligence Scale for Children – Fourth edition; WPPSI-III = Wechsler Preschool and Primary Scale of Intelligence – Third edition.

[2] MDI <70.

[3] Cognitive or language scales of <77.5 or WPPSI-III FSIQ <70.

included studies (3 out of 7) used multi-strain probiotics (Chou *et al.*, 2010; Firmansyah *et al.*, 2011; Jacobs *et al.*, 2017) and the remaining studies used single-strain probiotics (Akar *et al.*, 2017; Partty *et al.*, 2015; Sari *et al.*, 2012; Slykerman *et al.*, 2018). Only one study used milk containing a synbiotic composed of *Bifidobacterium longum* BL99, *Lactobacillus rhamnosus* LPR, inulin, fructooligosaccharides, and long chain polyunsaturated fatty acids as their intervention product (Firmansyah *et al.*, 2011).

Cognitive outcomes were assessed as mental development index (MDI) using BSID-II, cognitive and language scores using Bayley-III, full scale intelligence quotient (FSIQ) using Wechsler Preschool and Primary Scale of Intelligence – third edition (WPPSI-III) or Wechsler Intelligence Scale for Children – fourth edition (WISC-IV), Conners 3™ parent T-scores, Conners CPT 3™ T-scores, Cambridge Neuropsychological Test Automated Battery (CANTAB) scores, or diagnosis of ADHD or Asperger Syndrome (AS). Six out of the seven included studies further assessed the outcome in follow-up studies (Akar *et al.*, 2017; Chou *et al.*, 2010; Jacobs *et al.*, 2017; Partty *et al.*, 2015; Sari *et al.*, 2012; Slykerman *et al.*, 2018).

Of the 7 studies, only 1 reported a positive effect of probiotic supplementation on cognitive function. Partty *et al.* (2015) conducted a study with *L. rhamnosus* GG (LGG) supplementation compared to placebo, which was given to Finnish mothers for 4 weeks before delivery and continuously given for 6 months after delivery if they breastfed, or to the children (Partty *et al.*, 2015). At the age of 13, the effect on cognitive function was assessed as the proportion of ADHD or AS diagnosed by a child psychiatrist or neurologist. All children with a diagnosis of ADHD or AS were in the placebo group and none were in the probiotic group (P=0.008). Among 35 children in the placebo group, ADHD was diagnosed in 3 (8.6%) children, AS in 1 (2.8%) child, and both ADHD and AS in 2 (5.7%) children. Logistic regression analysis controlled for gender was performed, and the result remained significant (P=0.02). In this study, gut microbiota composition was also assessed using fluorescence *in situ* hybridisation (FISH) and quantitative polymerase chain reaction (qPCR) at the age of 3 weeks, 3, 6, 12, 18, 24 months, and 13 years. While it found a lower median number of *B. longum* at the age of 3 months and a lower mean number of cells belonging to genus *Bifidobacterium* at the age of 6 months among children who later developed ADHD or AS, no single microbiota composition could be constantly distinctive between children with or without ADHD or AS. Six remaining studies in this review found no significant difference in the cognitive outcome of probiotic supplementation in children and adolescents (Table 1).

4. Discussion

Within a limited amount of available RCTs, the favourable effect of probiotic supplementation on cognitive function in children and adolescents was only shown in one study using LGG administration with an outcome of risk reduction of developing ADHD or AS (i.e. autism) (Partty *et al.*, 2015). Other included RCTs with varying strains, durations of intervention, start-time of administration, and outcomes reported lack of observed benefits after probiotic supplementation. Metagenomic analyses on gut microbiota composition were not performed in any of these studies.

This is the first review to evaluate the effect of probiotic supplementation on cognitive function in children and adolescents. The review has carefully evaluated important information of the selected studies, such as randomisation and blinding procedures, and followed the necessary procedure in drawing final conclusion. Only randomised and double-blind controlled trials, which are the most rigorous way of determining a cause-effect relationship, were included in this review, thus adding strength to the review.

All included studies declared the application of randomisation and blinding procedure. Partty *et al.* (2015) conducted a double-blind, placebo-controlled study with computer-generated randomisation. The clinical diagnosis of ADHD and AS was established by a child psychiatrist or neurologist blinded to randomisation and not involved in the study. This diagnosis, which was made at the age of 13, seems to be in line with the recommendation from the American Academy of Pediatrics. It is mentioned that the evaluation for ADHD could be initiated for any child aged 4-18 years and children who meet the criteria for autism are often diagnosed starting from age 2 to 3 years (Harrington and Allen, 2014; Wolraich *et al.*, 2011). Although all the diagnosed children were male, the result remained significant after the logistic regression analysis was controlled for gender. Other clinical characteristics of the study population including birth weight and length were comparable between probiotic and placebo groups. However, this study was conducted in a high-income country. Hence, extrapolating this result for children in middle- and low-income countries might be more challenging because of the different living environments.

When probiotics were given to the children with ADHD and/or autism including AS, they constantly demonstrated potential benefits. Two previous studies of probiotic supplementation in autistic children reported positive effects on cognitive-related autistic symptoms (Kaluzna-Czaplinska and Blaszczyk, 2012; Shaaban *et al.*, 2018). However, these studies used the pre-post design and did not apply double-blind randomised controlled trial design. Shaaban *et al.* (2018) found significant improvement in autistic symptoms including speech/language/ communication and sensory/ cognitive awareness that were measured with the Autism

Treatment Evaluation Checklist (ATEC) before and after supplementation of a multi-strain potential probiotic containing *L. acidophilus, L. rhamnosus,* and *B. longum* for 3 months (Shaaban *et al.*, 2018). A study by Kaluzna-Czaplinska and Blaszczyk (2012) reported significant improvement in the ability to concentrate and carry out orders in a cohort study after probiotic therapy with *L. acidophilus* strain Rosell-11 for 2 months.

Previous studies have reported lower amounts of *Bifidobacterium,* higher amounts of *Sutterella wadsworthensis,* and subspecies of *Clostridia* in the stool of autistic children (Finegold, 2011; Finegold *et al.*, 2002; Liu *et al.*, 2019; Sandler *et al.*, 2000; Wang *et al.*, 2013; Williams *et al.*, 2012). The latter produces propionic acid which may have a role in the development of some forms of autism spectrum disorders (Stackebrandt and Rainey, 1997). In acidotic conditions (e.g. during 'sickness'), propionic acid becomes more lipid-soluble and concentrated within cells (Karuri *et al.*, 1993; Rorig *et al.*, 1996). Patients with inherited metabolic disorders, such as propionic acidemia, are unable to metabolise propionic acid and often present with regressive cognitive impairment (Macfabe, 2013; Wajner *et al.*, 2004). Interestingly, neurochemical changes caused by intraventricular propionic acid in the animal brain are also consistent with findings in autism spectrum disorders patients, including neuroinflammation, increased oxidative stress, mitochondrial dysfunction, glutathione depletion, and altered phospholipid/acylcarnitine profiles (MacFabe *et al.*, 2007, 2008, 2011). However, the study by Partty *et al.* (2015) found no constant distinctive feature of the microbiota composition in ADHD or AS development (Partty *et al.*, 2015). Other mechanisms of action of gut microbiota in the gut-brain axis might be active such as vagal pathways, in addition to the altered microbiota composition itself (Bonaz *et al.*, 2018). In addition, another study using LGG with shorter duration of supplementation showed potential effect on neurological outcome as measured by reduction of incidence of suboptimal *Hammersmith Neurological Infant Examination* score (Romeo *et al.*, 2011). This study also found a significant reduction in gastrointestinal colonisation by Candida among the probiotics group. This assumed that candidemia may also have a role in affecting neurological outcomes. However, due to different outcomes, it is not clear whether similar pathways are active in this study when applied over a longer period. It is also worth mentioning that metagenomic analysis to provide more complete information on gut microbiota composition had not been done in any of the included studies in this review (Wang *et al.*, 2015).

There are some notable issues of reviewing the effects of probiotics on cognitive function that warrant further investigation. The strain of probiotics which are capable of affecting cognition are still unclear. This could possibly be best explored by proteomic and transcriptomic studies in human tissue that are capable of identifying activated particular proteins and transcription factors in response to probiotic supplementation with a specific strain (Haider and Pal, 2013). For instance, the connectivity-map analysis from transcriptomic study in human small intestinal cells of volunteers ingesting LGG has shown the similar transcriptomes obtained between this strain and treatment with compound that controls apoptosis (Van Baarlen *et al.*, 2011), but has not yet mentioned the compound related to brain development (e.g. histone deacetylase inhibitor to up-regulate BDNF and GDNF gene transcription) (Wu *et al.*, 2008). Defining strain-specific effects in improving cognition may answer the lack of significant effects in spite of longer duration of probiotic supplementation during the golden period of brain and microbiota development (Shanahan, 2011). Furthermore, it is also necessary to provide data related to the mechanism of action of probiotics in the gut-brain axis, such as through metagenomic analysis of gut microbiota composition and changes in SCFAs concentration. The duration of supplementation in the included studies are also extensively varied. In about half of the included studies (4 out of 7), probiotics were administered only until hospital discharge (Table 1). Time of administration is also critical for cognitive outcomes, as it seems that any intervention is ideally administered within the first 1000 days of life when the first window opportunity of brain development takes place (Cusick and Georgieff, 2016).

Other issues that limit our understandings are related to the assessment of cognitive function. Cognition encompasses many aspects of thinking, such as attention, memory, and executive function (Hughes and Bryan, 2003). Cognitive impairment might also be manifested as ADHD and as part of autistic symptoms (Baron-Cohen, 2004; Faraone *et al.*, 2015). However, the outcome of ADHD or autism itself only illustrate a limited domain of cognition in children and adolescents. Future trials should account more comprehensive domain of cognitive evaluation with more innovative approaches conducted to assess earlier brain development such as foetal brain imaging (Griffiths *et al.*, 2017). It is also important to integrate the intervention with maternal factors, psychosocial stimulation, and dietary quality for improving cognitive function in children (Aboud and Yousafzai, 2019; Cusick and Georgieff, 2016; Prado *et al.*, 2017). Moreover, while the urge of early childhood optimisation is increasing, its benefits to cognition might need to be observed as a long-term outcome. Since developmental scores tend to be more stable after the age of two, a follow-up study of the longer-term outcome is necessary (Fernald *et al.*, 2017). A study by Colombo *et al.* (2013) found no difference in BSID scores at the age of 18 months after receiving a formula containing certain fatty acids in infancy but showed significant differences in vocabulary and IQ scores at ages 5-6 years (Colombo *et al.*, 2013). In our review, only two early childhood intervention studies assessed the longer term outcome at above 5 years of age (Partty *et al.*, 2015; Slykerman *et al.*, 2018). It is highly likely (e.g. through use

of antibiotics) that the effects of supplementation decrease over time due to the significant influence of environmental factors on childhood development (Bailey *et al.*, 2017; Fernald *et al.*, 2017). In this matter, it is compulsory to have several time points of observation in the follow-up study to provide more comprehensive results.

Although this review highlighted the potential effect of probiotics in reducing the risk of the development of ADHD or autism-related outcomes, included studies were limited to English publications and did not included unpublished literature such as theses/dissertations which may have excluded relevant studies. Nevertheless, probiotic might be a safe and effective therapy for autism-related syndromes including cognitive disturbance in clinical practice (Fattorusso *et al.*, 2019). Extensive preclinical and uncontrolled studies have demonstrated the benefits of probiotic supplementation in reducing gastrointestinal and behavioural symptoms of autism (Grossi *et al.*, 2016; Hsiao *et al.*, 2013; Kaluzna-Czaplinska and Blaszczyk, 2012; Parracho *et al.*, 2010; Shaaban *et al.*, 2018; West *et al.*, 2013). However, more randomised double-blind clinical trials of probiotic supplementation for ADHD or autism-related outcomes are still needed to identify its strain-specific efficacy before routine use.

5. Conclusions

A favourable effect of probiotic supplementation on cognitive function in children and adolescents was observed in one study with LGG supplementation by a risk reduction of developing ADHD or AS (i.e. autism). More comprehensive intervention involving maternal factors, psychosocial stimulation, and dietary quality should be performed. Innovative assessment of early brain development (e.g. foetal brain imaging) and long-term cognitive assessment (e.g. school performance) are warranted for future trials.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/BM2019.0068.

Table S1. Cochrane risk of bias summary.

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