What can the gut microbiome teach us about the connections between child physical and mental health? A systematic review

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ABSTRACT

A deeper understanding of the gut-brain axis is of significance in pediatrics, given the influential role of early childhood experiences and exposures in shaping the microbiome, and health, across the life course. This systematic review synthesized evidence on the connection between the gut microbiome and mental health in children with physical illness. Six electronic databases were systematically searched and data extracted according to the Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) guidelines. Of 1,476 identified articles, 11 articles reporting on 9 unique studies (all randomized controlled trials) were included. Most studies examined the gut microbiome in infants with colic, while the remaining studies investigated outcomes in children aged 1 day to 18 years at-risk for atopic dermatitis or irritable bowel syndrome. Baseline and post-intervention gut microbiome differences varied across studies. Findings on psychological functioning also varied, with only half of the captured studies showing a positive effect of intervention on psychological wellbeing. Only two studies analyzed the association between the gut microbiome and psychological outcomes; each with a different pattern of results. As the field moves forward, it will be critical to gain a better understanding of the microbiome characteristics that influence mental health outcomes in pediatric populations.

Keywords: Microbiome; mental health; physical health; chronic illness; childhood; quality of life.

INTRODUCTION

Children with critical or chronic illness can experience a range of stressors, including invasive treatments and side effects, periods of hospitalisation and prolonged medical care, changes to academic and social schedules, shifts in family dynamics, and fear and uncertainty about the future (Tapanes, Distelberg, Williams-Reade, & Montgomery, 2015). The multiple systems that children are embedded within are often disrupted during illness, altering the typical course of development, which can further exacerbate symptoms and their consequences (Wood, 1993).

Thus, it is unsurprising that many medical conditions are associated with poorer quality of life (QOL) and aberrant emotional and behavioral outcomes in children (Hysing, Elgen, Gillberg, Lie, & Lundervold, 2007; Hysing, Elgen, Gillberg, & Lundervold, 2009; Pinquart & Shen, 2011). Children with complex congenital heart disease, for example, have a heightened risk of neurodevelopmental impairment, internalizing and externalizing disorders, and poorer health-related QOL compared to healthy, typically-developing children (Denniss, Sholler, Costa, Winlaw & Kasparian, 2018; Kasparian, Winlaw & Sholler, 2016; Tesson et al., in press), and this pattern is found across a range of disease groups (Greenley et al., 2010; Mackner & Crandall, 2007; Varni, Limbers, & Burwinkle, 2007). While this has potentially negative implications for child wellbeing, it can also be viewed as an opportunity to enhance the psychosocial context and improve pediatric outcomes. Psychological interventions for children with functional gastrointestinal disorders, for example, have been shown to improve physical health outcomes (Brent, Lobato, & LeLeiko, 2009; Levy et al., 2010, 2013).

To further improve clinical care and QOL in pediatric populations, it is critical we develop a deeper understanding of the biological and psychosocial mechanisms underlying poor mental

health outcomes. One biological system receiving substantial attention from the fields of medicine and psychology is the gastrointestinal system. There is mounting evidence that the gut microbiome (i.e., the vast and diverse array of microorganisms residing in the gastrointestinal tract, and their collective genomes) contributes to both physical and mental health (Callaghan, 2017; Cho & Blaser, 2012; Cowan et al., 2018; Cowan & Richardson, in press; Dinan & Cryan, 2017; Kinross, Darzi, & Nicholson, 2011; Sarkar et al., 2018). Preclinical models provide causal evidence that the gut microbiome affects emotional development. Germ-free mice born and raised in the absence of any microorganisms, for example, exhibit atypical social and emotional behavior (Arentsen, Raith, Qian, Forssberg, & Heijtz, 2015; Clarke et al., 2012; Davis, Bryda, Gillespie, & Ericsson, 2016; Heijtz et al., 2011; Hoban et al., 2017), alongside physiological markers of anxiety, depression and stress reactivity (Erny et al., 2015; Luczynski et al., 2016; Neufeld, Kang, Bienenstock, & Foster, 2011; Sudo et al., 2004). Correlational studies in adult humans show that individuals with a range of physical and mental health difficulties – from depression and autism spectrum disorder to Parkinson's disease, irritable bowel syndrome (IBS), stroke and cancer – have different microbial profiles compared to healthy peers (Jiang et al., 2015; Parracho, Bingham, Gibson, & McCartney, 2005; Scheperjans et al., 2015; Schwabe & Jobin, 2013; Yin et al., 2015; Zheng et al., 2016).

In children, emerging research is identifying similar disruptions to the microbiome and gut development across a range of health conditions, including asthma, IBS, inflammatory bowel disease, cystic fibrosis, and infant colic (Johnson & Versalovic, 2012; Nielsen et al., 2016; O'Mahony, Stilling, Dinan, & Cryan, 2015; Slattery, MacFabe, & Frye, 2016). Studies investigating the link between the gut microbiome and neurodevelopmental outcomes are much more limited, but preliminary studies have observed associations between the early

microbiome and cognitive and emotional development in healthy infants (Carlson et al., 2018; Christian et al., 2015). Mounting research indicates gut microbiome composition is associated with autism, a neurodevelopmental disorder (Vuong & Hsiao, 2017). Such outcomes highlight the overlap between gut microbiome maturation and critical periods of neurodevelopment. Indeed, the instability and immaturity of the gut microbiome from infancy to adolescence means it is more vulnerable to environmental insults, such as antibiotic use, stress and infection, all of which may lead to dysbiosis and poorer neurodevelopmental outcomes (Clarke, Mahoney, Dinan, & Cryan, 2014; Codagnone et al., in press). Given many pediatric populations are exposed to such environmental insults, further research on how the microbiome affects brain development and mental health is of importance if we are to improve child outcomes.

Associations between the microbiome and physical and mental health are further supported by evidence that probiotics (i.e., beneficial bacterial strains) may improve physical symptoms and health-related QOL in pediatric populations (Guandalini et al., 2010; Horvath, Dziechciarz, & Szajewska, 2011; Sung et al., 2018); however, studies in this area often do not measure the direct impact of intervention on the gut microbiome. This represents an important gap in our understanding of the mechanisms underlying probiotic treatment effects which in turn hinders the implementation of such treatments in clinical practice due to uncertainties regarding who will benefit and under what circumstances. To clarify the current state of the science and shed light on potential microbiome mechanisms, this systematic review aimed to synthesize and critically appraise all available evidence on the connection between the gut microbiome and mental health in children with physical illness.

METHODS

Data search strategy and sources

The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, Altman, & Group, 2009) were used to identify, screen and extract data from identified articles. A systematic search was carried out in April 2018 using six electronic databases (MEDLINE, Embase, PsycINFO, the Cumulative Index to Nursing and Allied Health Literature, EMcare, Scopus). The search strategy was developed in MEDLINE and adapted for use in each database. A string of key terms was combined for each search, including: *child, medical illness, disease, microbiome, microbiota, dysbiosis, psychological stress, and quality of life* (search strategy available on request). Date range restrictions were not applied to maximize the number of articles returned. Ancestry methods, citation chaining and prolific author searching in Scopus were used to identify additional articles. Auto-alerts were created using the same unique search algorithm for each database, with findings incorporated into the review until June 30, 2018.

Study selection criteria

Studies were eligible for review if they: (i) Examined infants, children, or young people aged 0-18 years with a physical health condition; (ii) Measured the composition or diversity of the gut microbiome or its constituents; and (iii) Assessed mental health or illness, QOL, or any form of psychosocial functioning of the child or young person, as determined by parent- or self-report (e.g., frequency of crying in infants). Studies examining outcomes in both children and adults were included only if child outcomes were reported separately. Articles were excluded if they investigated non-human animals, reported on qualitative data, or were

systematic reviews, case studies, conference abstracts, dissertations, commentaries or editorials, or published in a non-English language.

Initially, titles were screened by one author (JK) to determine eligibility. Abstracts and full-texts were independently screened for eligibility by two authors (JK, NAK). Conflicts of opinion were resolved through consensus or consultation with a third reviewer (CYO). Article full-texts deemed eligible for review (or for which eligibility could not be determined from the title or abstract) were retrieved for data extraction. While review articles were excluded from analysis, these were collected and screened to ensure originality of the review concept. No published reviews of a similar nature to the present study were identified.

Data extraction

Data extraction was undertaken by two authors (JK, CSMC) using a standardized data collection form, and checked for accuracy by a third author (CYO). Extracted data included: study characteristics (e.g., authors, country, research design), sample characteristics (e.g., sample size, age, medical condition), study methods (e.g., techniques and measures to assess the gut microbiome and psychosocial functioning) and results, as relevant to the review. Results of captured studies were then synthesized and presented using a narrative approach.

Risk of bias analysis

Risk of bias was independently assessed by two authors (JK, NAK) using the Cochrane Collaboration Tool for Assessing Risk of Bias (Higgins et al., 2011). For each study, potential sources of bias were rated across six domains (selection, performance, detection, attrition, reporting, other bias), with risk categorized as ‘high’, ‘unclear’ or ‘low’.

RESULTS

Database searching yielded 1,476 articles. After removing duplicates, 1,122 articles were eligible for title and abstract screening, with 1,026 articles not meeting eligibility criteria. Full-text screening was carried out for 96 articles, and reference chaining performed, yielding a total of 11 articles reporting on 9 unique studies for inclusion (Figure 1).

Study characteristics

Overall, the unique studies included 602 participants, with sample sizes ranging from 9-167 participants, and children ranging in age from 1 day to 18 years. Publication dates ranged from 2008 to 2018, and all captured studies either reported the findings of a randomized controlled trial (RCT), or analysis of fecal samples and psychosocial data collected as part of an RCT (Table 1). Captured studies were undertaken in Europe (*n*=7), the United States (*n*=2), or Australia (*n*=2). Most articles (*n*=9; 82%) presented outcomes in infants with colic, with one study investigating the prevention of atopic dermatitis in infants, and one focused on children and adolescents with IBS.

Risk of bias

Overall, risk of bias was low or unclear in the captured studies (Table 2); however, risk of selective reporting bias (81%) and risk of attrition bias (63%) was high or unclear most trials. Allocation concealment and blinding of participants and outcomes generally posed low risk. Five trials were registered and one referenced a protocol. No study reported on missing data handling and small sample sizes were common.

Microbiota interventions and analyses

Almost all interventions involved probiotic supplementation, with the exception of one trial that tested a prebiotic (psyllium fiber) supplementation (Table 3). Approximately half of the probiotic studies (*n*=6) assessed *Lactobacillus (L.) reuteri* DSM 17938; the remaining studies used a variety of probiotic preparations, including multi-strain formulations (*n*=2), another single strain (*L. rhamnosus GG* ATCC 53103, *n*=1), and a synbiotic treatment (i.e., combination of probiotics and prebiotics, *n*=1). Studies varied in treatment duration (2 weeks to 6 months), and approach to microbiome analysis, including quantitative polymerase chain reaction (qPCR; *n*=8), culture-based techniques (*n*=2), and fluorescence in situ hybridization (FISH; *n*=1) to measure specific taxa and terminal restriction fragment length polymorphism (T-RFLP; *n*=2) or 16S ribosomal RNA sequencing using 454 pyrosequencing (*n*=3) to examine overall microbiome diversity or composition.

Effects of intervention on the gut microbiota of infants with colic

Baseline differences. Of the three studies that included a healthy control group, pre-treatment comparisons indicated differences in the microbiota of babies with colic. Lower levels of *Bifidobacterium* (Pärtty, Lehtonen, Kalliomäki, Salminen, & Isolauri, 2015), increased relative abundance of gram-negative anaerobes (Mentula, Tuure, Koskenala, Korpela, & Könönen, 2008), and increased prevalence of *Escherichia [E.] coli* and other indole-positive coliforms (i.e., *Klebsiella oxytoca;* Mentula et al., 2008; Savino, Garro, Montanari, Galliano, & Bergallo, 2018) were found in babies with colic. Mentula et al. (2008) also found several (typically low-abundance) aerobic genera were undetectable in colicky infants (*Streptococcus*, *Micrococcus*, *Corynebacterium*, *Bacillus* spp. and yeasts).

Probiotic detection. Following treatment, four studies tested for the presence of the specific probiotic species in participant fecal samples. In three studies of *L. reuteri,* the strain was not detected in any untreated participants and was detectable in 45% to 92% of treated infants (Nation et al., 2017; Savino et al., 2010; Sung et al., 2014). In another study using a multi-strain probiotic (Mentula et al., 2008), one of the major components of the formulation (*L. rhamnosus* GG) was detected in high concentrations in the feces of all probiotic-treated infants (*n*=5).

Targeted analyses. Targeted culture- or PCR-based analyses of specific taxa of interest focused mainly on lactobacilli and bifidobacteria as examples of beneficial taxa and *E. coli* as an example of a putative pathogen (although *E. coli* Nissle 1917 strain is considered probiotic; Wassenaar, 2016). Generally, lactobacilli and/or bifidobacteria were increased after intervention (Mentula et al., 2008; Pärtty et al., 2015; Savino et al., 2010; Savino et al., 2018), with one exception (Baldassarre et al., 2018), where only a trend for increased lactobacilli was observed (p =0.053). Results for *E. coli* were less consistent; studies reported decreased levels in the intervention group (Savino et al., 2010), no difference between intervention and control groups (Savino et al., 2018; Sung et al., 2014), or increased rates of colonization in infants who received the probiotic (Nation et al., 2017). Enterococci (which include common commensal species) were found to be increased in one study (Mentula et al., 2008), while another study found no change in levels of *Clostridium butyricum* (Savino et al., 2010).

Whole microbiome analyses. In terms of intervention effects on the overall gut microbiota of babies with colic, no differences between treatment and control conditions were observed in the five studies measuring alpha diversity (Fatheree et al., 2017; Nation et al., 2017; Sung et

al., 2014), beta diversity (Roos et al., 2013), or total bacterial load (Baldassarre et al., 2018). Fatheree et al. (2017) reported some post-treatment differences between groups at the family and genus levels, but no statistical analyses or beta-diversity plot were provided.

Functional analyses. Two studies measured the functional output of the microbiome. Comparing samples from infants with colic to controls, Mentula et al. (2008), observed slightly elevated levels of unsaturated and branched fatty acids, and low levels of certain saturated and hydroxy cellular fatty acids, but no differences in fermentation products (i.e., gases and short-chain fatty acids). After intervention, the majority of probiotic-treated infants exhibited increased acetic acid and lactic acid production as well as reduced gaseous hydrogen production, while none of these parameters changed in placebo controls (the small sample size precluded statistical analysis). Using proton nuclear magnetic resonance, Baldassarre et al. (2018) also observed differential changes in the fecal metabolomics profile of probiotic-treated infants with colic; however, none of these metabolites were altered in the same way across studies (only acetate was altered by probiotic intervention in both, but in opposite directions), and neither study attempted to link the functional microbiome profile with infant outcomes.

Effects of intervention on the gut microbiota of children with other medical conditions

The two non-colic studies tested different interventions; one synbiotic (2 probiotic strains plus a prebiotic) for preventing atopic dermatitis (Roze et al., 2012), and one prebiotic (psyllium fiber) for IBS (Shulman et al., 2017). In the prebiotic study, overall microbiome composition did not change across the treatment period for the intervention or control group, regardless of the taxonomic level examined (from phylum down to operational taxonomic unit; Shulman et al., 2017). In the synbiotic study, culture- and qPCR-based analyses

revealed lower levels of staphylococci and higher levels of lactobacilli in the synbiotic group after one month of treatment. These effects were maintained at 6 months (i.e., end of treatment), although only at the trend level for lactobacilli. There was also a trend towards increased bifidobacteria in the synbiotic-treated infants at 6 months (Roze et al., 2012).

Mental health measures and outcomes

***Infant colic*.** The main outcomes in all studies of infant colic were changes in frequency of crying from baseline to intervention completion and follow-up, and amount of daily crying at a specified time-point between intervention and control groups. Crying data in two of the included studies were not collected independently, but were derived from larger trials. Specifically, results reported by Roos et al. (2013) were the same as those reported by Savino et al. (2010) and were therefore not included in this synthesis of crying outcomes. Nation et al. (2017) also assessed crying data included in an earlier publication (Sung et al., 2014), but the results of both studies have been included here because the data were analyzed differently in each case.

All studies measured crying by asking parents to record daily crying time across the study period using a diary. One study also interviewed parents at intervention completion to estimate daily crying (Pärtty et al., 2015). Only four studies referenced use of a validated diary of infant crying (Fatheree et al., 2017; Pärtty et al., 2015; Savino et al., 2018; Sung et al., 2014). Two studies (Fatheree et al., 2017; Sung et al., 2014) also recorded infant fussing time, which was defined as “behavior that is not quite crying but not awake and content either” (Barr, Kramer, Boisjoly, McVey-White, & Pless, 1988). Two studies also assessed infant or family QOL (Baldassarre et al., 2018; Sung et al., 2014).

Across studies, there were no significant differences in baseline crying between intervention and control groups, and all studies reported a decrease in daily crying or fussing time in both groups across the study period. There were, however, mixed results in terms of whether probiotic intervention affected crying. Three studies reported no differences between treatment and placebo control groups (Fatheree et al., 2017; Mentula et al., 2008; Nation et al., 2017), and three studies reported a significant reduction in crying time in infants receiving probiotic treatment compared to the placebo (Baldassarre et al., 2018; Pärtty et al., 2015; Savino et al., 2010; Savino et al., 2018). In terms of colic symptoms, two studies reported significant, but conflicting, differences between probiotic and placebo groups. Pärtty et al. (2015) found mothers reported a reduction in infant daily crying during their interview, but not when using the diary. Sung et al. (2014), somewhat surprisingly, found infants in the probiotic group fussed more than the placebo group at treatment end, although this difference was no longer present at 6-month follow-up and was not observed for crying. Study subgroup analyses revealed that the detrimental effect of probiotics was limited to formula-fed infants, whereas no treatment effect was observed in breast-fed infants.

Of the two studies that assessed QOL, one found that probiotic intervention improved family QOL, as measured on a 10-point scale (Baldassarre et al., 2018), whereas the other found no differences on a more comprehensive, validated measure of child health-related QOL (Sung et al., 2014).

Atopic dermatitis and irritable bowel syndrome. Like the colic studies, the study of infants with atopic dermatitis assessed daily crying time using a structured parent diary (Roze et al., 2012) and found that infants who received a synbiotic supplement exhibited significantly less crying and more quiet behavior in the 3 days prior to a 1-month clinical visit (during

intervention); however, these differences were no longer observed at 6-months (post-intervention), likely due to low levels of crying in both groups. Shulman et al. (2017) conducted a more comprehensive assessment of QOL and emotional and behavioral wellbeing in children with IBS; however, assessments were carried out at baseline only, so differences between children receiving psyllium fiber versus placebo were not tested.

Associations between the gut microbiota and mental health outcomes

Only two studies reported statistical analyses attempting to link the microbiota with psychosocial outcomes, both in the context of infant colic. Roos et al. (2013) found an increase in the abundance of Bacteroidetes over the treatment period in responders (infants with >50% reduction in colic symptoms) compared to non-responders. While not necessarily treatment-related, a higher proportion of probiotic-treated infants were classified as 'responders'. In the second study, Nation et al. (2017) found a positive correlation between *L. reuteri* colonization density and crying time in the subset of infants colonized by the bacterium.

Effect of intervention on other physical health outcomes

Across the captured RCTs, a range of physical outcomes were monitored, including pain, number of bowel movements, markers of inflammation in plasma, fecal calprotectin, presence of atopic dermatitis, and height and weight. In general, no significant differences were observed between intervention and control groups across the study period, with a few exceptions. First, prophylactic synbiotic treatment reduced risk for atopic dermatitis, although the significance of this effect was reduced to the trend level after adjustment for birth mode and family history of allergic disease (Roze et al., 2012). Second, prebiotic psyllium fiber reduced pain frequency, but not severity, compared to placebo in children with IBS (Shulman

et al., 2017). Third, Savino et al. (2018) reported elevated levels of fecal calprotectin in infants with colic compared to healthy controls at baseline, which declined over the intervention period only in the probiotic treatment group - although this was the only study (of five) to report an effect on fecal calprotectin. No negative treatment-associated effects were reported across the studies.

DISCUSSION

Research investigating the gut microbiome has increased exponentially in recent years, with studies showing that the microorganisms within our gastrointestinal tract can have important implications for human health and wellbeing. Generally, literature on the gut microbiome suggests complex and dynamic associations between microbiota and our physical and mental health. At the crossover of microbiome, physical, and mental health research, there is some evidence from that the microbiota contributes to mental health outcomes in adult medical populations (particularly with IBS; Kajander et al., 2008; Silk, Davis, Vulevic, Tzortzis, & Gibson., 2009; Yoon et al., 2013). This systematic review aimed to determine whether there is empirical evidence to directly support this association in pediatric populations, and to shed light on the associations between the microbiome and mental health in children with physical illness.

Overall, we identified only 11 studies examining both the microbiome and psychological functioning in infants, children or young people with a medical condition. Unexpectedly, most of the identified studies examined infants, focused on one of only three physical health conditions, and all were clinical trials targeting the gut microbiome with either a prebiotic (psyllium fiber), probiotic, or synbiotic supplementation. Generally, no robust or consistent pattern emerged from the studies identified. Gut microbiome differences at baseline and

following intervention varied across studies, depending on the physical health condition and type of analysis conducted. Compared to healthy controls, studies showed that infants with colic have an atypical microbial profile (Mentula et al., 2008; Pärtty et al., 2015; Savino et al., 2018), including lower levels of *Bifidobacterium* (Pärtty et al., 2015), consistent with the extant literature (de Weerth, Fuentes, Puylaert, & de Vos, 2013; Rhoads et al., 2009). Intervention had no effect on measures of the whole microbiome in infants with colic or IBS (Baldassarre et al., 2018; Fatheree et al., 2017; Nation et al., 2017; Roos et al., 2013; Shulman et al., 2017; Sung et al., 2014), although this may be at least partly attributable to the shallow depth of resolution in analyses. Targeted analyses of lactobacilli and/or bifidobacteria suggested that the interventions enriched these beneficial taxa (Mentula et al., 2008; Pärtty et al., 2015; Savino et al., 2010, 2018), while results were mixed for *E.coli* (Nation et al., 2017; Savino et al., 2010, 2018; Sung et al., 2014), which often acts as a pathogen. Findings on psychosocial functioning also varied, with only about half of the captured studies showing a positive effect of intervention on measures of infant distress (namely, crying).

The main question this review sought to address was whether there is evidence that gut microbiota are associated with mental health outcomes in children with physical illness. We identified only two studies examining microbiome-mental health associations (Nation et al., 2017; Roos et al., 2013), demonstrating that research investigating the role of the microbiome in pediatric physical and mental health is extremely limited (Bai, Behera, & Bruner, 2018). Although the findings of these studies are of interest - with one set of results showing a link between beneficial *Bacteroidetes* and greater reductions in infant crying across the intervention period (Roos et al., 2013) - the evidence is clearly preliminary and precludes firm conclusions or clinical recommendations. Thus, while the gut microbiome has exciting

potential to teach us more about the links between child physical and mental health, the findings of this review show much research is needed to substantiate these associations.

Limitations of captured studies and the current review

Some of the variability in findings across studies may be explained by microbiome factors, as well as differences in study design, intervention, measures, and time-points analyzed. Although most studies collected and analyzed fecal samples at baseline, analyses were generally not sufficiently powered or detailed to allow identification of potential pre-existing individual differences that might predict treatment response. As an example, one of the interventions (psyllium fiber) was previously shown to act as a prebiotic only in individuals with low baseline levels of bifidobacteria (Elli, Cattivelli, Soldi, Bonatti, & Morelli, 2008). Many of the microbiome and psychological analyses were limited by small sample sizes and single points of data collection (usually at treatment completion). These factors likely contributed to the paucity of data on interactions between the microbiome and psychological outcomes. Differences in baseline microbiome composition and microbiome response to treatment (e.g., colonization by introduced probiotic species) are factors that may contribute to individual differences in physical and mental health outcomes, as observed in two studies within the present dataset (Nation et al., 2017; Roos et al., 2013), as well as in other cases (e.g., microbiome modulation of cancer therapy efficacy; Bashiardes, Tuganbaev, Federici, & Elinav, 2017; Gopalakrishnan, Helmink, Spencer, Reuben, & Wargo, 2018).

Importantly, a number of studies included participants who varied across factors known to be key determinants of infant microbiome composition, including birth mode (vaginal vs. Cesarean section; Chu et al., 2017; Dominguez-Bello et al., 2010) and diet (breast- vs. formula feeding; Ho et al., 2018), and generally these variables were not accounted for in

analyses. These factors are particularly important in the studies of infants with colic, given the effects of birth mode are most potent in early life (Chu et al., 2017; Hill et al., 2017) and there is now evidence that formula feeding reduces the efficacy of probiotic interventions for colic (Sung et al., 2018).

Further, the techniques used to assess the microbiome in most of the captured studies were outdated. Most studies relied on culture-based methods or qPCRs targeting selected bacterial taxa, and only three studies used 16S rRNA sequencing, which is generally considered the current minimum standard for microbiome studies. In fact, the field is rapidly moving towards the use of shotgun metagenomics sequencing (Jovel et al., 2016; Ranjan, Rani, Metwally, McGee, & Perkins, 2016), a technique not used in any of the captured studies. Shotgun sequencing is more expensive than 16S but provides superior resolution for profiling of the microbiome down to the species, and even the strain, level. In contrast, 16S is currently reliable only to the genus level, making it difficult to differentiate beneficial from pathogenic taxa. If the aim of conducting microbiome analyses is to differentiate disease or treatment profiles and identify the functional relevance of any observed changes, then much greater specificity is needed. The need for in-depth analyses is supported by the findings of the current set of studies, where differences in the representation of specific species were observed using targeted culture-based techniques and qPCR, while metabolomics and short-chain fatty acid analyses hinted at differences in the functional capacity of the microbiome after intervention.

Like the microbiome assessments, measures used to assess mental health or psychological functioning in the captured studies were limited, and no studies specifically assessed mental health outcomes such as anxiety or depression. Of the studies that assessed crying as a

measure of colic symptoms and infant distress, only about half referenced using a validated diary, making it difficult to assess data quality in the remaining studies. Other measures lacked specificity, with one study using only a single 10-point scale to assess family QOL. While many psychometric measures ask participants for retrospective or general accounts of factors such as QOL, mood and anxiety, for studies reliant on accurate daily accounts of behavior (e.g., crying), it is critical to improve the quality and specificity of measures so as to minimize error and recall bias.

This review had several limitations. First, the heterogeneity of methodologies used across the captured studies made it difficult to pool results and precluded meta-analysis. Second, there was an overrepresentation of infant colic, limiting the generalizability of findings, and making it difficult to draw conclusions on the current state of the microbiome and mental health outcomes in other medical conditions. Likewise, most of captured studies focused on medical conditions of a non-critical or acute (rather than chronic) nature, and investigated outcomes in infants, meaning the results presented here may not generalize to critical or chronic medical conditions, nor to older children and adolescents.

Future research recommendations

Overall, there is much room for improvement regarding the design, analysis, and interpretation of studies investigating the role of the microbiome in child physical and mental health. To elucidate the functional role of the microbiome in this field, recommendations for future studies include: (i) consistent use of more detailed and robust microbiome analyses, including contemporary sequencing techniques (i.e., 16S and shotgun metagenomics), (ii) use of advanced computational and statistical modeling, (iii) stronger justification for treatment length, dosage, and probiotic strain used, (iv) use of validated instruments to measure mental

health and psychological functioning, and (v) larger sample sizes and specific statistical analyses of interactions between the microbiome and mental health outcomes. This will likely require strong interdisciplinary collaboration between microbiome experts (microbiologists, bioinformaticians), clinicians (e.g., psychologists, physicians), and researchers specializing in mental health and psychobiological development.

This review highlights the large gap in our knowledge of the connections between the gut microbiome and physical and mental health in pediatric populations – with this gap being even larger for children and young people with critical or chronic medical illness. Pleasingly, efforts are underway to investigate the effect of probiotic treatment on both the microbiome and health-related QOL in children with chronic conditions, such as cystic fibrosis (Coffey, Garg, Homaira, Jaffe, & Ooi, 2018). Our group is also investigating associations between the gut microbiome, HPA axis, and neurobiological and psychological factors in children and adolescents with complex congenital heart disease.

CONCLUSIONS

As the field moves forward, it will be critical to gain a better understanding of the baseline microbiome characteristics that can influence treatment (e.g., deficiencies in particular species) and mental health outcomes in pediatric populations. This would fit with the current push towards using the microbiome to inform and enhance personalized medicine approaches (Jobin, 2018; Kuntz & Gilbert, 2017; Petrosino, 2018). Targeting the gut microbiome during early development could have critical implications for disease prevention and management, and set in motion a lifetime of benefits for the child.

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Figures, tables, and captions

Figure 1. PRISMA diagram illustrating the systematic search process.

Table 1. Summary of article characteristics (*N*=11). All captured studies used a randomized controlled trial (RCT) design.

Table 2. Risk of bias in randomized controlled trials, rated using the Cochrane Risk of Bias tool. For each domain, studies were rated as having a high (-), low (+), or unclear (?) risk of bias using the Cochrane Risk of Bias tool.

Table 3. Summary of key findings across all captured articles (*N*=11), as relevant to this review.