Old Herborn University Seminar Monograph 35: Microbes impacting mammalian systems biology.
Editors: Peter J. Heidt, Tore Midtvedt, Andreas Schwiertz, James Versalovic, and Volker Rusch.
Old Herborn University Foundation, Herborn, Germany: 37-53 (2023).

EARLY-LIFE GUT MICROBIOTA AND NEURODEVELOPMENTAL OUTCOMES

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SUMMARY

Over the last decades, studies have revealed that the gut microbiota has much wider effects on host physiology and development than originally believed, including the modulation of brain development and behaviour. Recent insights into the complexity and magnitude of the human microbiota and its wide-ranging impact on health and disease have given way to a paradigm shift in our conceptualization of the origin of human brain disorders. Evidence from preclinical research, cross-sectional clinical studies, and preliminary microbiota-targeted intervention studies implicates the gut microbiota as a potential key susceptibility factor in neurodevelopmental and psychiatric disorders, including autism spectrum disorder (ASD). However, the microbiota-gut-brain axis field is nascent, and further investigations are needed to unravel the precise mechanisms mediating the intricate microbiota-host interactions and the potential of microbiota-based therapeutic strategies for these conditions. This article briefly highlights recent findings implicating the microbiota-gut-brain axis in the neurobiology of ASD, and novel metabolite-based therapeutic approaches to manage ASD. The emerging roles of the bacterial peptidoglycan signalling pathway in early life gut-brain communication are also presented.

INTRODUCTION

It is now well recognized that environmental influences during early life can profoundly affect the formation and later function of neural brain circuits, a phenonomen termed developmental programming. One such external environmental factor is the gut microbiota – the trillions of microorganisms inhabiting the gastrointestinal (GI) tract, including bacteria, archaea, fungi, and viruses – that through evolution has adapted to coexist in a mutualistic relationship with mammals (*Davenport* et al., 2017). During birth and rapidly thereafter, the new-born is massively colonized with microbes via vertical transmission from mother to infant (*Dominguez-Bello* et al., 2019; *House* et al., 2016). This postnatal microbial colonization process contributes to the developmental programming of epithelial barrier function, gut homeostasis, and angiogenesis, as well as promoting the development of the immune system (*Hooper* et al., 2012). A rapidly expanding body of research has revealed that the gut microbiota exerts a broader range of effects on host physiology and development beyond the GI tract, including the early life programming of brain circuits involved in the control of the stress response, motor activity, and cognitive functions (for a review, see: *Cryan* et al., 2019). Preclinical studies

using germ-free (GF) animals have shown that gut microbiota influences a wide-range of neurodevelopmental processes, including the maturation and function of microglia, primary resident immune cells of the brain (*Erny* et al., 2015; *Thion* et al., 2018), synaptogenesis (*Diaz Heijtz* et al., 2011), blood-brain barrier (BBB) formation and integrity (*Braniste* et al., 2014) and myelination (*Hoban* et al., 2016), as well as complex behaviours (*e.g.*, social and anxiety-like behaviours) (*Clarke* et al., 2013; *Diaz Heijtz* et al., 2011; *Neufeld* et al., 2011). Other studies have shown that gut microbiota influences behavioural abnormalities and brain pathologies observed in numerous animal models of neurodevelopmental and psychiatric disorders (*Lum* et al., 2020; *Needham* et al., 2018; *Sgritta* et al., 2019; *Tabouy* et al., 2018). One important lesson that has emerged from these preclinical studies is that many effects of the gut microbiota on the brain are sex-dependent (*Jaggar* et al., 2020). These studies also indicate that there are sensitive periods in early life during which the gut microbiota plays a critical role in shaping neural circuit formation and function.

In humans, the maturation of the gut microbiota occurs during the first years of postnatal life, occurring in parallel with critical neurodevelopmental processes (*e.g.*, synaptogenesis, myelination, and synaptic pruning) and the onset of neurodevelopmental disorders such as autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD) (for a review, see: *Borre* et al., 2014; *Diaz Heijtz*, 2016). It is increasingly acknowledged that neurodevelopmental and psychiatric disorders are often co-morbid with GI problems and an altered gut microbiota (*Cryan* et al., 2020). In addition, a growing number of preclinical and epidemiological studies suggest that early-life antibiotic exposure, that reduces microbiota diversity, could have detrimental effects on neurodevelopmental trajectories, and subsequently be a contributing factor to behavioural and psychological disturbances emerging in childhood (*Neuman* et al., 2018; *Otten* et al., 2022). The current challenge is to translate findings from animal models into humans, and to untangle the complex and dynamic interactions between the microbiota and the developing brain.

THE GUT MICROBIOTA AND AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) is an early-onset neurodevelopmental disorder, defined by the presence of social communication and interaction challenges in conjunction with restricted, repetitive behaviours and atypical sensory processing (*Lord* et al., 2018). The worldwide prevalence rate of ASD is currently estimated to be 1% (*Zeidan* et al., 2022). Manifestations of ASD are heterogenous, reflecting a spectrum of symptoms that may include individuals with intellectual disability and limited language ability, as well as individuals with average- or above-average intellectual abilities and typical language development. Moreover, many children with ASD experience GI and immune dysfunction, as well as a range of co-occurring somatic and psychiatric conditions including sleep disorders, epilepsy, and anxiety (*Hsiao*, 2014; *Leader* et al., 2022; *Madra* et al., 2020; *Zeidan* et al., 2022). For instance, several studies have shown that GI symptoms, such as abdominal pain, diarrhoea, constipation, and flatulence, are more common in children with ASD than their

neurotypically developing peers (*Chaidez* et al., 2014) and are positively associated with the severity of behavioural problems such as irritability, aggression, and repetitive behaviours (*Adams* et al., 2011; *Chakraborty* et al., 2021; *Wang* et al., 2011). Almost two decades ago, a small open-label study observed that treatment with oral vancomycin (a non-absorbable antibiotic that is active against Gram-positive bacteria) resulted in short-term benefit in a small group of children with regressive-onset autism (*Sandler* et al., 2000). Although antibiotics are not a suitable intervention strategy for the management of ASD, this study was among the first to suggest a link between the gut microbiota and ASD. Since then, multi cross-sectional studies have also reported an altered gut microbiota composition in children and adolescents with ASD, with lower gut bacterial diversity and an underrepresentation of beneficial bacteria (*e.g.*, Bifidobacterium species), along with an increased abundance of potentially pathogenic bacteria *Desulfovibrio* and *Clostridium* genera (*Andreo-Martinez* et al., 2022; *Bezawada* et al., 2020; *Iglesias-Vazquez* et al., 2020; *Xu* et al., 2019). There is, however, little consensus regarding the magnitude of changes or the existence of a specific microbial signature associated with ASD. It is worth mentioning that most of these studies were performed several years after children received the diagnosis of ASD and did not adequately record detailed dietary information or potential medical use. A recent study by Yap and colleagues provided evidence that ASD-associated gut microbiota changes can be attributed largely to a lack of dietary diversity (*Yap* et al., 2021). It is well-known that children with ASD often exhibit selective eating patterns (*Bourne* et al., 2022), and that they are more frequently exposed to antibiotics during the first years of life (*House* et al., 2016), which can adversely impact the gut microbiota (*Otten* et al., 2022). Nevertheless, the causal potential of the gut microbiota in ASD has been supported by preclinical studies including genetic (*e.g.*, Shank3B mutant mice) environmental (*e.g.*, valproic acid, GF, and maternal high-fat diet) and idiopathic rodent models of ASD (BTBR T+ Itpr3tf/J mice) (for a review, see: Alamoudi et al., 2022). Further support has been gathered from studies transferring the faecal gut microbiota from autistic children into GF mice, resulting in behavioural and molecular changes relevant to this condition (*Sharon* et al., 2019). However, it remains unclear whether the gut microbiota contributes to, or modifies the likelihood of ASD onset before any dietary changes occur. Indeed, a recent multi-omics analysis has highlighted the limitations of cross-sectional cohort studies (*Morton* et al., 2023), thus advocating for prospective, longitudinal multi-omics studies in combination with comprehensive patient metadata to elucidate the role of the gut microbiota in ASD.

EARLY-LIFE MICROBIOME DIFFERENCES IN INFANTS AT HIGH FAMILIAL RISK FOR ASD

ASD is now understood to be a multifactorial neurodevelopmental condition, involving genetic susceptibility, environmental risk factors, and gene-environmental interactions. One such risk factor is having a sibling with ASD, with studies consistently demonstrating a higher prevalence among siblings and in families with a history of ASD (*Lord* et al., 2018). Moreover, previous

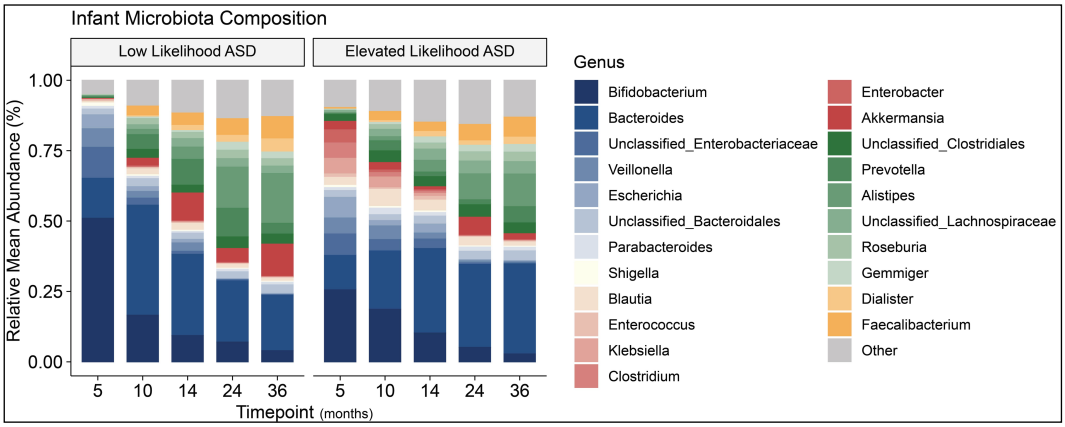

Figure 1: Gut microbiota composition level of infants at low likelihood (LL) and elevated likelihood (EL) of ASD from 5 to 36 months of age.
The relative mean abundance of the top taxa was calculated using the aggregated OTUs at the genus level. The largest differences between the two groups were observed at 5 months of age. The LL group harboured more Bifidobacteria (51% vs. 26%), while the EL group had more Clostridium (5% vs. 0.005%) compared to the LL group (Reprinted with permission from *Zuffa* et al., 2023)

investigations have shown that about 50% of younger siblings of children with ASD develop in an atypical manner, approximately 20% develop ASD, and another 30% show delays or deficits in other areas of development or behaviour (*Ozonoff* et al., 2014). Using a prospective longitudinal study design, Zuffa and colleagues recently studied the developmental profile of the faecal microbiota and metabolome in infants with and without a family history of ASD (in first- or second-degree relatives) across the first 3 years of life (*Zuffa* et al., 2023), a critical period when the gut microbiota and brain are both undergoing rapid development (*Borre* et al., 2014).

At 5 months of age, this study found pronounced alterations in the gut microbiota composition of infants at elevated likelihood (EL) of developing ASD (*i.e.*, siblings of children with ASD) compared to low-likelihood (LL) infants (*i.e.*, infants without a family history of ASD) (see Figure 1). Specifically, infants in the EL group harboured less beneficial *Bifidobacterium* species (*B. bifidum, B. longum* and *B. kashiwanohense*) and more *Clostridium* (*C. bolteae*, *C. difficile*, *C. clostridioforme*, *C. neonatale)* species compared to infants in the LL group. Members of the genus *Bifidobacterium* are among the first colonizers of the neonatal human gut and have been associated with various health benefits (*e.g.*, folate production, protection against pathogens and development of the immune system). On the other hand, *Clostridium* species, which have previously been linked to ASD, are considered pathobionts and responsible for inflammation when homeostasis is disrupted (*Lees* et al., 2016).

Untargeted metabolic profiling highlighted that infants in the LL group excreted greater amounts of faecal γ-aminobutyric acid (GABA) GABA, which progressively declined with age (*Zuffa* et al., 2023). In contrast, infants in the EL group did not display similar developmental changes, with GABA being consistently low across the first 36 months of life (*Zuffa* et al., 2023). Furthermore, integrated microbiome-metabolome analysis revealed a strong positive correlation between the

identified *Bifidobacterium* species and GABA, as well as negative associations with *Clostridium* species (*Zuffa* et al., 2023). These observations raised the possibility that competition between *Clostridium* and *Bifidobacterium* species may exist and lead to reduced availability of GABA in the infant gut. To test this hypothesis, Zuffa and colleagues used a simplified *in vitro* model with different ratios of the identified *Bifidobacterium* and *Clostridium* species in the presence of GABA and its metabolic precursors (*Zuffa* et al., 2023). Fascinatingly, *in vitro* experiments revealed that *Bifidobacterium species* can produce GABA while *Clostridium* species can consume it, indicating that a delicate balance exists between these bacteria genera in the infant gut, with consequences for the bioavailability of GABA and its modulatory effects on the infant host.

The general development level including gross motor functions, visual perception, fine motor skills, receptive language, and expressive language of infants in the EL and LL groups was evaluated using the Mullen Scales of Early Learning (MSEL) test at 5 and 36 months of age and with Autism Diagnostic Observation Schedule Second Edition (ADOS-2; an assessment tool for the diagnosis and evaluation of ASD) at 36 months of age. Consistent with prior literature, no significant developmental changes were observed between infants in the EL and LL groups at 5 months (*Gamliel* et al., 2007). At 36 months, however infants in the EL group had lower MSEL and ADOS-2 compared to infants in the LL group. Although none of the infants were diagnosed with ASD at 36 months of age, more subtle presentations of this condition might still be diagnosed later in development, when daily functional demands increase (*e.g.*, in the school setting). Moreover, other co-occurring somatic and psychiatric conditions may not be evident at this early age. Despite this limitation, this study clearly demonstrated that alterations in gut microbiota composition and functionality of infants at EL of ASD occur before any atypical development could be detected at 36 months of age (*Zuffa* et al., 2023), supporting a possible role of the gut microbiota in emerging behavioural variability later in life.

Emerging evidence suggests that the development of the infant gut microbiota is influenced by host genetics, but that this effect is subtle and affects only certain bacteria species (*Palmeira* et al., 2022). The absence of major confounding perinatal risk factors such as preterm birth and antibiotic exposure in the above cohort of infants suggest a role for host genetic risk factors in the observed early-life differences in gut microbiota composition and functionality of infants in the EL group. Interestingly, recent studies have shown that many high-confidence risk genes for ASD are expressed not only in the developing brain, but also in the developing gut (*Niesler* and *Rappold*, 2021). Moreover, recent preclinical studies have shown that mutations in some of these genes (*e.g.*, Shank3B mutant) alter the enteric nervous system, leading to abnormal GI motility and structural changes in the GI tract (for a review, see: *Alamoudi* et al., 2022). These findings suggest that genetic variants previously linked to behavioural symptoms in ASD could contribute to the development of an atypical gut microbiota due to alterations in the host gut environment.

POTENTIAL ROLE OF BACTERIAL-DERIVED GABA IN NEURODEVELOPMENT

GABA acts as the main inhibitory neurotransmitter in the central nervous system (CNS) and is significantly involved in a wide range of physiological and psychological processes. Alterations in the central GABAergic system have been implicated in the neurobiology of several neurodevelopmental and psychiatric disorders, including ASD (for a review, see: *Zhao* et al., 2021). Early in life, however, GABAergic synaptic transmission is excitatory, and plays an important role in brain development (*Ganguly* et al., 2001). GABAergic dysfunction disturbs the optimal excitatory/inhibitory (E/I) balance in the brain, altering the integrity and function of neural circuits. Indeed, an imbalance in E/I neurotransmission ratio in cortical networks has been suggested as a plausible neurobiological pathway underlying cognitive symptomatology in ASD (*Marenco* et al., 2018). Over the past decades, studies have shown that several commensal gut bacteria produce neurotransmitters such as dopamine, histamine, serotonin, and GABA. Several members of the genus *Bifidobacterium*, such as *Bifidobacterium dentium*, *Bifidobacterium longum* subsp. infantis and *Bifidobacterium adolescentis* have been shown to produce GABA (*Hidalgo-Cantabrana* et al., 2017). It remains unclear whether bacterial-derived GABA present in systemic circulation enters the brain. For instance, Luck and colleagues showed that GF mice mono-colonized with *Bifidobacterium dentium,* which possesses the enzymatic machinery to produce GABA from glutamate, glutamine, and succinate, displayed increases in faecal GABA concentrations, but no changes within the brain (*Luck* et al., 2021). Nonetheless, there is evidence that the central GABA system can be modulated via the vagus nerve and enteric nervous system (ENS). In a landmark study by Bravo and colleagues, it was demonstrated that ingestion of *Limosilactobacillus reuteri,* a probiotic with anti-inflammatory properties, can regulate emotional behaviour and central GABA receptor expression in mice via the vagus nerve (*Bravo* et al., 2011). Other animal studies have implicated GABA producing species such as *Bifidobacterium infantis* in the early-life programming of the immune system (*Henrick* et al., 2021), raising the possibility that bacterial-derived GABA may indirectly modulate brain development and subsequent behaviour via multiple pathways.

NEW MICROBIOME-BASED THERAPEUTIC APPROACHES TO MANAGE ASD

There is growing evidence from animal studies that direct metabolites of the gut microbiota or products of the combinatorial metabolism between the microbiota and host (so called "neuroactive microbial metabolites") can cross the BBB (*Swann* et al., 2020), and directly modulate neural networks involved in the control of affective, social, and cognitive processes (*Needham* et al., 2022). In humans, several neuroactive gut-bacterial-derived metabolites such as 4-ethylphenyl sulphate (4EPS), p-cresyl sulphate (pCS), and other structurally related phenolic molecules, were elevated in faeces and plasma of individuals with ASD (*Needham* et al., 2021; *Zheng* et al., 2021). Moreover, preclinical studies using mouse models of ASD indicate that 4EPS is likely to contribute

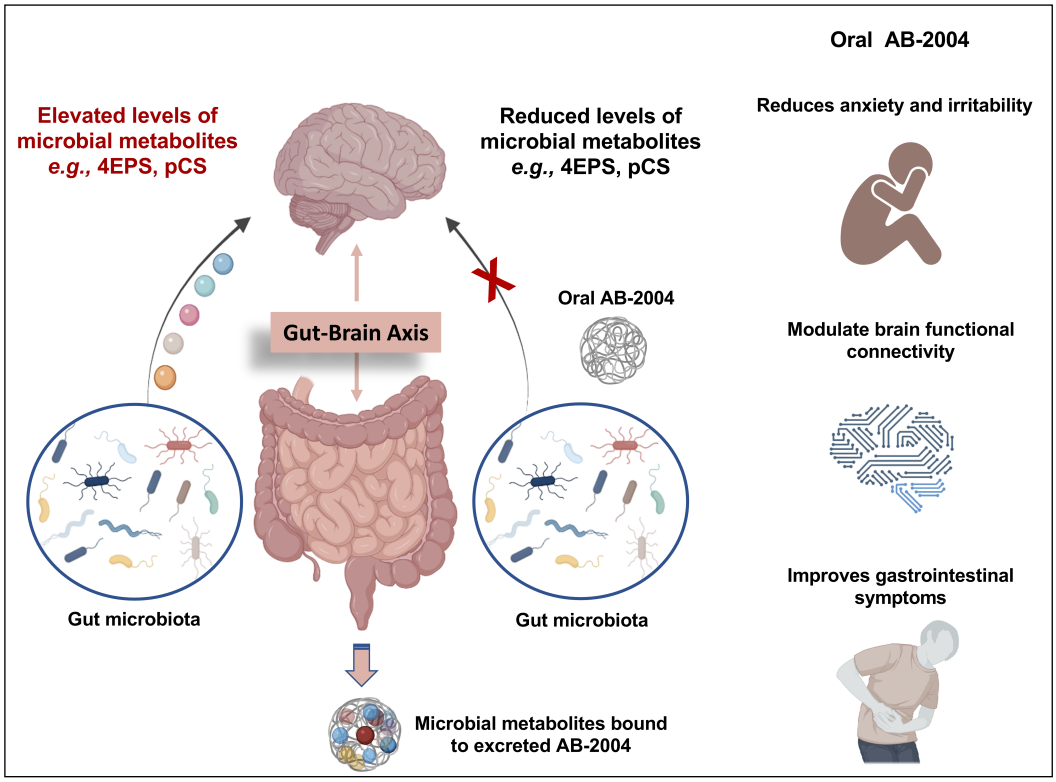

Figure 2: Treatment with AB-2004 improves gastrointestinal problems, brain functional connectivity and non-behavioural symptoms of ASD. **(**Modified from *Diaz Heijtz* et al., 2022).
Several biological pathways of communication along the gut-brain axis have been implicated, including the production of gut-bacterial-derived metabolites (so called neuroactive microbial metabolites) that can cross the blood brain barrier, and directly modulate the brain. Individuals with autism spectrum disorder (ASD) show elevated amounts of various neuroactive microbial metabolites such as 4-ethylphenyl sulphate (4EPS) and p-cresyl (pCS) in faeces and plasma. AB-2004 directly targets these metabolites in the gut, diminishing systemic exposure and limiting their impact on the brain.

to atypical neurodevelopment and GI dysfunction in mammals (*Hsiao* et al., 2013). Consequently, metabolite-based therapeutics targeting metabolites of the gut microbiota have emerged as an attractive new therapeutic approach to manage ASD. As such, rather than targeting the altered gut microbiota composition in ASD, this approach directly targets dysregulated neuroactive microbial metabolites in the gut, diminishing systemic exposure and limiting their impact on the brain. In a recent study, Campbell and colleagues provided the first preliminary clinical evidence that AB-2004, a first-in-class therapeutic that targets neuroactive microbial metabolites in the gut, can help reduce anxiety and irritability in adolescents with ASD, as well as GI problems (*Campbell* et al., 2022). AB-2004 is a spherical carbon absorbent that has high affinity for uremic toxins and related aromatic metabolites such as 4EPS, pCS, 3-indoxyl sulphate and hippurate, and therefore sequesters these molecules in the gut, preventing their absorption into the systemic circulation (see Figure 2). The results from an ongoing double-blinded placebo-controlled trial will reveal whether AB-2004 treatment proves to be effective for the manage-

ment of non-core behavioural symptoms and GI problems of ASD.

Other approaches have been employed to manipulate directly and profoundly the gut microbiota using faecal microbiota transplantation (FMT), as well as more subtle nutritional strategies (*e.g.*, psychobiotic diet, postbiotics) aimed at fortifying gut homeostasis and its microbial environment. Some preliminary FMT studies have shown positive preliminary results in ameliorating both autistic traits and GI symptoms (*Kang* et al., 2017; 2019; 2020). Successful examples of nutritional strategies have largely been limited to animal models of ASD. Current evidence supporting beneficial effects and long-term safety of microbiota-based interventions in infants at EL of ASD and young children with ASD are still limited.

THE MICROBIOTA-GUT-BRAIN AXIS, MATERNAL GUT MICROBIOTA AND NEURODEVELOPMENT

It has long been known that a complex bidirectional communication network exists between the gut and the CNS, known as the gut-brain axis, which includes the sympathetic and parasympathetic branches of the autonomic nervous system, the enteric nervous system and neuroendocrine and neuroimmune pathways. In recent decades, the gut microbiota has been identified as a crucial "third component" in the gut-brain crosstalk, thus leading to the current concept of the microbiota-gut brain axis. Communication along this axis involves multiple direct and indirect pathways, including neuronal circuits (*e.g.*, bidirectional vagus nerve-to-brain communication and the enteric nervous system), immune (*e.g.*, cytokines, monocyte trafficking), endocrine (*e.g.*, cortisol), and microbial-derived metabolites such as bacterial fermentation by-products such as short-chain fatty acids (SCFAs; propionate, butyrate, and acetate) (for a review, see: *Cryan* et al., 2019). However, the precise molecular mechanisms underlying the interactions between the gut microbiota and the developing brain remain to be elucidated.

It is now widely recognized that the maternal-foetal environment plays an important role in foetal brain and long-term neurodevelopmental trajectories, including susceptibility to neurodevelopmental and psychiatric disorders in childhood and adulthood (for a review, see: *Al-Haddad* et al., 2019). In recent years, the maternal gut microbiota has been associated not only with maternal health during pregnancy, but also with general foetal health and subsequent neurodevelopmental outcomes (for a review, see: *Bolte* et al., 2022). The developing foetus receives nutrients and other bioactive compounds from the maternal circulation, which could be profoundly influenced by the composition of the maternal gut microbes. Using GF mice, Braniste and colleagues investigated the impact of microbiota on the development of the BBB, which begins to develop early in foetal life and continues to mature during early postnatal life (*Braniste* et al., 2014). The authors found that GF mice displayed increased BBB permeability beginning in foetal life and continuing into adulthood, thus supporting a role of the maternal gut microbiota in BBB formation and maturation. The same study demonstrated that the SCFA butyrate could restore the integrity of the BBB in adult GF mice. Similarly, Erny and colleagues showed that a mix of SCFAs could also restore microglial malformation and immatu-

rity in adult GF mice (*Erny* et al., 2017). Recently, Kimura and colleagues demonstrated that SCFAs derived from the maternal gut microbiota could be translocated into the foetus and sensed through G protein-coupled receptors such as GPR41 in the developing sympathetic nervous system (*Kimura* et al., 2020). Crucially, they also showed that maternal-derived SCFAs are essential for the protection against high-fat-diet-induced metabolic syndrome and obesity in the offspring later in life (*Kimura* et al., 2020), thus highlighting the importance of the maternal gut microbiota in neurodevelopment. In another study, Vuong and colleagues recently showed that maternal microbial-derived metabolites shape foetal brain development and subsequent function (*Vuong* et al., 2020) and that this occurs in the absence of environmental challenges. These authors demonstrated that the maternal gut microbiota regulates metabolites (*e.g.*, trimethylamine-N-oxide, and hippurate), not only in the maternal serum, but also in the brains of the foetal offspring, promoting foetal thalamocortical axonogenesis (*Vuong* et al., 2020). These findings underscore the need to further characterize the influence that maternal microbial-derived metabolites or components have on the prenatal brain development of offspring and whether specific neural circuits in the developing brain are more sensitive to their modulation.

EMERGING ROLES OF THE BACTERIAL PEPTIDOGLYCAN SIGNALLING PATHWAY IN NEURODEVELOPMENT AND BEHAVIOUR

Traditionally, the translocation of bacterial cell wall components such as peptidoglycan (PGN, also called murein) motifs into the brain has mainly been considered in the context of a compromised BBB function (for example, bacterial or viral infections). Recent scientific discoveries revealing a previously unappreciated complexity of the human microbiota and its wide-ranging impact on health and disease have triggered a re-evaluation of our views about host-microbe interactions. The gut microbiota contains trillions of indigenous bacteria producing a diverse "peptidoglycome" that can disseminate systemically and reach peripheral organs (*Wheeler* et al., 2023). PGN is a unique and essential component of the bacterial cell wall that is absent in eukaryotes, which consists of glycan strands of two alternating β-1,4-linked sugars (N-acetylglucosamine and N-acetylmuramic acid) cross-linked by short peptides, containing two to five amino acids. It has highly dynamic structure that continuously undergoes remodelling, causing PGN motifs to be shed into the environment (*e.g.*, upon bacterial growth, replication, or death), a process termed PGN turnover. In mammals, PGN motifs are recognized by cytosolic NOD-like receptors (nucleotide-binding domain leucine-rich repeat containing receptors; Nod1 and Nod2) and PGN recognition proteins (PGRPs, Pglyrp1-4) (for a review see: *Mukherjee* et al., 2019; *Royet* et al., 2011). In a landmark study, Clarke and colleagues showed that the indigenous gut microbiota is a source of PGN which is translocated from the intestinal mucosa into circulation in the absence of pathogens (*Clarke* et al., 2010). Specifically, these authors showed that meso-diaminopimelic acid (meso-DAP)-type PGN, which is present in Gram-negative bacteria, could be translocated from the intestinal mucosa into neutrophils residing in bone marrow (*Clarke* et al., 2010).

Activation of the cytoplasmic pattern recognition receptors (PRR) Nod1 by meso-DAP-type PGN was sufficient to prime and restore neutrophil function in the bone marrow of mice with a manipulated microbiota (*i.e.*, GF or antibiotic-treated mice), revealing a previously undescribed role for PGN in priming systemic innate immunity in the absence of infection (*Clarke* et al., 2010).

In 2017, Arentsen and colleagues demonstrated that PGN fragments derived from the indigenous gut microbiota can be translocated into the developing brain and sensed by specific pattern recognition receptors (PRRs) of the innate immune system (*Arentsen* et al., 2017). Importantly, PGN levels in the brain increase in parallel with postnatal microbial colonization processes (*Arentsen* et al., 2017). Other groups have independently confirmed that PGN motifs from gut microbiota translocate across the intestinal barrier and can reach multiple peripheral organs, including the brain (*Wheeler* et al., 2023). Using expression-profiling techniques, Arentsen and colleagues also demonstrated that two families of PRRs that specifically detect PGN (*i.e.,* PGRPs and NOD-like receptors) and the PGN transporter PepT1 are highly expressed in the developing brain during specific windows of postnatal development in a brain-region and sex-dependent manner (*Arentsen* et al., 2017). Moreover, they showed that the absence of PGN-recognition protein 2 (Pglyrp2) leads to alterations in the expression of the autism risk genes c-Met and BDNF (brain-derived neurotrophic factor), which are implicated in the formation and modulation of brain circuits. Furthermore, juvenile Pglyrp2 knockout (KO) mice display immediate strong motivation to interact with same-sex novel social stimulus, without any alterations in anxiety-like behaviours or motor activity. Fascinatingly, this behavioural phenotype was more pronounced in male juvenile offspring. In another study, the same authors showed that the absence of Pglyrp2 leads to major sex-dependent alterations in motor and anxiety-like behaviour later in life (*Arentsen* et al., 2018). In this case, Pglyrp2 KO female mice, but not males, display increased levels of anxiety-like behaviour. However, they have better motor performance. These observations suggest that the modulatory effects of Pglyrp2 in the brain are highly dependent upon multiple host factors including age, sex, and domain-specific circuits in the brain. In an elegant study, Gabanyi and colleagues recently demonstrated that muramyl dipeptide (MDP)-type PGN (which is present in both Gram-positive and Gram-negative bacteria) regulates appetite and body temperature via activation of Nod2-expressing GABAergic neurons in the female hypothalamus (*Gabanyi* et al., 2022). Consistent with earlier studies by Arentsen and colleagues, these authors showed that Nod2 KO female mice, but not males, developed anxiety-like behaviour and metabolic dysregulation later in life (*Gabanyi* et al., 2022). Other studies have shown that selective deletion of Nod1 in intestinal epithelial cells increases the susceptibility of mice to stress-induced anxiety-like behaviour and cognitive impairment (*Pusceddu* et al., 2019). Taken together, these findings underscore the need to unravel the bacteria PGN pathways across different stages of brain development. In parallel, the potential influence of this pathway in typical and atypical human brain development could be explored by taking advantage of recent advances in brain organoid technologies.

CONCLUSIONS AND PERSPECTIVES

The gut microbiota is now recognized as a key regulator of brain development and behaviour, and a potential susceptibility factor in neurodevelopmental and psychiatric disorders such as ASD. Increasing evidence from both preclinical and clinical studies indicates that common perinatal risk factors (*e.g.*, stress, altered diet and antibiotic exposures) can perturb the maturation of the infant gut microbiota, with potential adverse consequences for early-life gut-brain interactions. However, it remains unclear whether the gut microbiota plays a role in the aetiology of neurodevelopmental disorders such as ASD. A recent preliminary study of infants at EL of ASD has provided strong evidence supporting the role of the gut microbiota on behavioural variability emerging around three years of age. There is an urgent need for multi-centre prospective longitudinal studies of infants at elevated risk for ASD from pregnancy until the first postnatal years of life, coupled with comprehensive metadata (*e.g.*, genetic information of infants and their families, dietary habits, perinatal risk factors, and relevant clinical outcomes) and multi-omics approaches.

The first years of postnatal life represent a period of rapid changes in brain structure and function, with the neonatal brain growing from about 36% to about 80-90% of its volume. During this period, the developing neural circuits in the brain undergo major changes, including massive outgrowth of dendrites and axons, rapid formation of new synapses (synaptogenesis), expansion of glial cells, and myelination. The striking increase in brain growth observed during this period, occurring in parallel with the maturation of the infant gut microbiota, indicates that this is not only a critical time-window of developmental vulnerability, but also a time of opportunity in which therapeutic interventions may have a maximal effect on neural circuits and subsequent functions. Consequently, there is a great need to unravel the precise molecular mechanisms underlying the communication between the gut microbiota and the developing brain to develop more targeted interventions for infants and young children at risk for neurodevelopmental and psychiatric disorders.

Recent studies have identified the bacterial PGN signalling pathway as a potential key regulator of gut microbiota–brain interactions in early life. From a clinical perspective, it would be important to explore potential associations between the PGN signalling pathway (from genetic variants in PGN-sensing molecules to dysregulation of the peptidoglycome) and neurodevelopmental disorders.

ACKNOWLEDGMENTS

Work from the laboratory of R.D.H. cited in this review has been supported by grants from the Swedish Research Council, The Swedish Brain Foundation, the Olle Engkvist Byggmästare Foundation, and the Frimurare Barnhus Foundation.

LITERATURE

Adams, J.B., Johansen, L.J., Powell, L.D., Quig, D., and Rubin, R.A.: Gastrointestinal flora and gastrointestinal status in children with autism--comparisons to typical children and correlation with autism severity. BMC Gastroenterol. 11, 22 (2011).

Al-Haddad, B.J.S., Oler, E., Armistead, B., Elsayed, N.A., Weinberger, D.R., Bernier, R., Burd, I., Kapur, R., Jacobsson, B., Wang, C., Mysorekar, I., Rajagopal, L., and Adams Waldorf, K.M.: The fetal origins of mental illness. Am. J. Obstet. Gynecol. 221, 549-562 (2019).

Alamoudi, M.U., Hosie, S., Shindler, A.E., Wood, J.L., Franks, A.E., and Hill-Yardin, E.L.: Comparing the Gut Microbiome in Autism and Preclinical Models: A Systematic Review. Front. Cell. Infect. Microbiol. 12, 905841 (2022).

Andreo-Martinez, P., Rubio-Aparicio, M., Sanchez-Meca, J., Veas, A., and Martinez-Gonzalez, A.E.: A Meta-analysis of Gut Microbiota in Children with Autism. J. Autism. Dev. Disord. 52, 1374-1387 (2022).

Arentsen, T., Khalid, R., Qian, Y., and Diaz Heijtz, R.: Sex-dependent alterations in motor and anxiety-like behavior of aged bacterial peptidoglycan sensing molecule 2 knockout mice. Brain Behav. Immun. 67, 345-354 (2018).

Arentsen, T., Qian, Y., Gkotzis, S., Femenia, T., Wang, T., Udekwu, K., Forssberg, H., and Diaz Heijtz, R.: The bacterial peptidoglycan-sensing molecule Pglyrp2 modulates brain development and behavior. Mol. Psychiatry 22, 257-266 (2017).

Bezawada, N., Phang, T.H., Hold, G.L., and Hansen, R.: Autism Spectrum Disorder and the Gut Microbiota in Children: A Systematic Review. Ann. Nutr. Metab. 76, 16-29 (2020).

Bolte, E.E., Moorshead, D., and Aagaard, K.M.: Maternal and early life exposures and their potential to influence development of the microbiome. Genome Med. 14, 4 (2022).

Borre, Y.E., O'Keeffe, G.W., Clarke, G., Stanton, C., Dinan, T.G., and Cryan, J.F.: Microbiota and neurodevelopmental windows: implications for brain disorders. Trends Mol. Med. 20, 509-518 (2014).

Bourne, L., Mandy, W., and Bryant-Waugh, R.: Avoidant/restrictive food intake disorder and severe food selectivity in children and young people with autism: A scoping review. Dev. Med. Child Neurol. 64, 691-700 (2022).

Braniste, V., Al-Asmakh, M., Kowal, C., Anuar, F., Abbaspour, A., Toth, M., Korecka, A., Bakocevic, N., Ng, L.G., Kundu, P., Gulyás, B., Halldin, C., Hultenby, K., Nilsson, H., Hebert, H., Volpe, B.T., Diamond, B., and Pettersson,S.: The gut microbiota influences blood-brain barrier permeability in mice. Sci. Transl. Med. 6, 263ra158 (2014).

Bravo, J.A., Forsythe, P., Chew, M.V., Escaravage, E., Savignac, H.M., Dinan, T.G., Bienenstock, J., and Cryan, J.F.: Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc. Natl. Acad. Sci. USA 108, 16050-16055 (2011).

Campbell, A.S., Needham, B.D., Meyer, C.R., Tan, J., Conrad, M., Preston, G.M., Bolognani, F., Rao, S.G., Heussler, H., Griffith, R., Guastella, A.J., Janes, A.C., Frederick, B., Donabedian, D.H., and Mazmanian, S.K.: Safety and target engagement of an oral small-molecule sequestrant in adolescents with autism spectrum disorder: an open-label phase 1b/2a trial. Nat. Med. 28, 528-534 (2022).

Chaidez, V., Hansen, R.L., and Hertz-Picciotto, I.: Gastrointestinal problems in children with autism, developmental delays or typical development. J. Autism Dev. Disord. 44, 1117-1127 (2014).

Chakraborty, P., Carpenter, K.L.H., Major, S., Deaver, M., Vermeer, S., Herold, B., Franz, L., Howard, J., and Dawson, G.: Gastrointestinal problems are associated with increased repetitive behaviors but not social communication difficulties in young children with autism spectrum disorders. Autism 25, 405-415 (2021).

Clarke, T.B., Davis, K.M., Lysenko, E.S., Zhou, A.Y., Yu, Y., and Weiser, J.N.: Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. Nat. Med. 16, 228-231 (2010).

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R.D., Shanahan, F., Dinan, T.G., and Cryan, J.F.: The microbiome-gut-brain axis during early life regulates the

hippocampal serotonergic system in a sex-dependent manner. Mol. Psychiatry 18, 666-673 (2013).

Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, M., Codagnone, M.G., Cussotto, S., Fulling, C., Golubeva, A.V., Guzzetta, K.E., Jaggar, M., Long-Smith, C.M., Lyte, J.M., Martin, J.A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'Connor, R., Cruz-Pereira, J.S., Peterson, V.L., Rea, K., Ritz, N.L., Sherwin, E., Spichak, S., Teichman, E.M., van de Wouw, M., Ventura-Silva, A.P., Wallace-Fitzsimons, S.E., Hyland, N., Clarke, G., and Dinan, T.G.: The Microbiota-Gut-Brain Axis. Physiol. Rev. 99, 1877-2013 (2019).

Cryan, J.F., O'Riordan, K.J., Sandhu, K., Peterson, V., and Dinan, T.G.: The gut microbiome in neurological disorders. Lancet Neurol. 19, 179-194 (2020).

Davenport, E.R., Sanders, J.G., Song, S.J., Amato, K.R., Clark, A.G., and Knight, R.: The human microbiome in evolution. BMC Biol. 15, 127 (2017).

Diaz Heijtz, R.: Fetal, neonatal, and infant microbiome: Perturbations and subsequent effects on brain development and behavior. Semin. Fetal Neonatal Med. 21, 410-417 (2016).

Diaz Heijtz, R., Gressens, P., and Swann, J.R.: Targeting microbial metabolites to treat autism. Nat. Med. 28, 448-450 (2022).

Diaz Heijtz, R., Wang, S., Anuar, F., Qian, Y., Bjorkholm, B., Samuelsson, A., Hibberd, M.L., Forssberg, H., and Pettersson, S.: Normal gut microbiota modulates brain development and behavior. Proc. Natl. Acad. Sci. USA 108, 3047-3052 (2011).

Dominguez-Bello, M.G., Godoy-Vitorino, F., Knight, R., and Blaser, M.J.: Role of the microbiome in human development. Gut 68, 1108-1114 (2019).

Erny, D., Hrabe de Angelis, A.L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., Keren-Shaul, H., Mahlakoiv, T., Jakobshagen, K., Buch, T., Schwierzeck, V., Utermöhlen, O., Chun, E., Garrett, W.S., McCoy, K.D., Diefenbach, A., Staeheli, P., Stecher, B., Amit, I., and Prinz, M.: Host microbiota constantly control maturation and function of microglia in the CNS. Nat. Neurosci. 18, 965-977 (2015).

Erny, D., Hrabe de Angelis, A.L., and Prinz, M.: Communicating systems in the body: how microbiota and microglia cooperate. Immunology 150, 7-15 (2017).

Gabanyi, I., Lepousez, G., Wheeler, R., Vieites-Prado, A., Nissant, A., Chevalier, G., Wagner, S., Moigneu, C., Dulauroy, S., Hicham, S., Polomack, B., Verny, F., Rosenstiel, P., Renier, N., Gomperts Boneca, I., Eberl, G., and Lledo, P.-M.: Bacterial sensing via neuronal Nod2 regulates appetite and body temperature. Science 376, eabj3986 (2022).

Gamliel, I., Yirmiya, N., and Sigman, M.: The development of young siblings of children with autism from 4 to 54 months. J. Autism Dev. Disord. 37, 171-183 (2007).

Ganguly, K., Schinder, A.F., Wong, S.T., and Poo, M.: GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. Cell 105, 521-532 (2001).

Henrick, B.M., Rodriguez, L., Lakshmikanth, T., Pou, C., Henckel, E., Arzoomand, A., Olin, A., Wang, J., Mikes, J., Tan, Z., Chen, Y., Ehrlich, A.M., Bernhardsson, A.K., Mugabo, C.H., Ambrosiani, Y., Gustafsson, A., Chew, S., Brown, H.K., Prambs, J., Bohlin, K., Mitchell, R.D., Underwood, M.A., Smilowitz, J.T., German, J.B., Frese, S.A., and Brodin, P.: Bifidobacteria-mediated immune system imprinting early in life. Cell 184, 3884-3898 (2021).

Hidalgo-Cantabrana, C., Delgado, S., Ruiz, L., Ruas-Madiedo, P., Sanchez, B., and Margolles, A.: Bifidobacteria and Their Health-Promoting Effects. Microbiol. Spectr. 5 (2017).

Hoban, A.E., Stilling, R.M., Ryan, F.J., Shanahan, F., Dinan, T.G., Claesson, M.J., Clarke, G., and Cryan, J.F.: Regulation of prefrontal cortex myelination by the microbiota. Transl. Psychiatry 6, e774 (2016).

Hooper, L.V., Littman, D.R., and Macpherson,

A.J.: Interactions between the microbiota and the immune system. Science 336, 1268-1273 (2012).

House, S.A., Goodman, D.C., Weinstein, S.J., Chang, C.H., Wasserman, J.R., and Morden, N.E.: Prescription Use among Children with Autism Spectrum Disorders in Northern New England: Intensity and Small Area Variation. J. Pediatr. 169, 277-283 (2016).

Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow, J., Reisman, S.E., Petrosino, J.F., Patterson, P.H., and Mazmanian, S.K.: Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 155, 1451-1463 (2013).

Hsiao, E.Y.: Gastrointestinal issues in autism spectrum disorder. Harv. Rev. Psychiatry 22, 104-111 (2014).

Iglesias-Vazquez, L., Van Ginkel Riba, G., Arija, V., and Canals, J.: Composition of Gut Microbiota in Children with Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Nutrients 12, 792 (2020).

Jaggar, M., Rea, K., Spichak, S., Dinan, T.G., and Cryan, J.F.: You've got male: Sex and the microbiota-gut-brain axis across the lifespan. Front. Neuroendocrinol. 56, 100815 (2020).

Kang, D.W., Adams, J.B., Gregory, A.C., Borody, T., Chittick, L., Fasano, A., Khoruts, A., Geis, E., Maldonado, J., McDonough-Means, S., Pollard, E.L., Roux, S., Sadowsky, M.J., Schwarzberg Lipson, K., Sullivan, M.B., Caporaso, J.G., and Krajmalnik-Brown, R.: Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome 5, 10 (2017).

Kang, D.W., Adams, J.B., Coleman, D.M., Pollard, E.L., Maldonado, J., McDonough-Means, S., Caporaso, J.G., and Krajmalnik-Brown, R.: Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. Sci. Rep. 9, 5821 (2019).

Kang, D.W., Adams, J.B., Vargason, T., Santiago, M., Hahn, J., and Krajmalnik-Brown, R.: Distinct Fecal and Plasma Metabolites in Children with Autism Spectrum Disorders and Their Modulation after Microbiota Transfer Therapy. mSphere 5, e00314-20 (2020).

Kimura, I., Miyamoto, J., Ohue-Kitano, R., Watanabe, K., Yamada, T., Onuki, M., Aoki, R., Isobe, Y., Kashihara, D., Inoue, D., Inaba, A., Takamura, Y., Taira, S., Kumaki, S., Watanabe, M., Ito, M., Nakagawa, F., Irie, J., Kakuta, H., Shinohara, M., Iwatsuki, K., Tsujimoto, G., Ohno, H., Arita, M., Itoh, H., and Hase, K.: Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. Science 367, eaaw8429 (2020).

Leader, G., Abberton, C., Cunningham, S., Gilmartin, K., Grudzien, M., Higgins, E., Joshi, L., Whelan, S., and Mannion, A.: Gastrointestinal Symptoms in Autism Spectrum Disorder: A Systematic Review. Nutrients 14, 1471 (2022).

Lees, E.A., Miyajima, F., Pirmohamed, M., and Carrol, E.D.: The role of Clostridium difficile in the paediatric and neonatal gut - a narrative review. Eur. J. Clin. Microbiol. Infect. Dis. 35, 1047-1057 (2016).

Lord, C., Elsabbagh, M., Baird, G., and Veenstra-Vanderweele, J.: Autism spectrum disorder. Lancet 392, 508-520 (2018).

Luck, B., Horvath, T.D., Engevik, K.A., Ruan, W., Haidacher, S.J., Hoch, K.M., Oezguen, N., Spinler, J.K., Haag, A.M., Versalovic, J., and Engevik, M.A.: Neurotransmitter Profiles Are Altered in the Gut and Brain of Mice Mono-Associated with Bifidobacterium dentium. Biomolecules 11, 1091 (2021).

Lum, G.R., Olson, C.A., and Hsiao, E.Y.: Emerging roles for the intestinal microbiome in epilepsy. Neurobiol. Dis. 135, 104576 (2020).

Madra, M., Ringel, R., and Margolis, K.G.: Gastrointestinal Issues and Autism Spectrum Disorder. Child Adolesc. Psychiatr. Clin. N. Am. 29, 501-513 (2020).

Marenco, S., Meyer, C., van der Veen, J.W., Zhang, Y., Kelly, R., Shen, J., Weinberger, D.R., Dickinson, D., and Berman, K.F.: Role

of gamma-amino-butyric acid in the dorsal anterior cingulate in age-associated changes in cognition. Neuropsychopharmacol. 43, 2285-2291 (2018).

Morton, J.T., Jin, D.M., Mills, R.H., Shao, Y., Rahman, G., McDonald, D., Zhu, Q., Balaban, M., Jiang, Y., Cantrell, K., Gonzalez, A., Carmel, J., Frankiensztajn, L.M., Martin-Brevet, S., Berding, K., Needham, B.D., Zurita, M.F., David, M., Averina, O.V., Kovtun, A.S., Noto, A., Mussap, M., Wang, M., Frank, D.N., Li, E., Zhou, W., Fanos, V., Danilenko, V.N., Wall, D.P., Cárdenas, P., Baldeón, M.E., Jacquemont, S., Koren, O., Elliott, E., Xavier, R.J., Mazmanian, S.K., Knight, R., Gilbert, J.A., Donovan, S.M., Lawley, T.D., Carpenter, B., Bonneau, R., and Taroncher-Oldenburg, G.: Multi-level analysis of the gut-brain axis shows autism spectrum disorder-associated molecular and microbial profiles. Nat. Neurosci. 26, 1208-1217 (2023).

Mukherjee, T., Hovingh, E.S., Foerster, E.G., Abdel-Nour, M., Philpott, D.J., and Girardin, S.E.: NOD1 and NOD2 in inflammation, immunity and disease. Arch. Biochem. Biophys. 670, 69-81 (2019).

Needham, B.D., Tang, W., and Wu, W.L.: Searching for the gut microbial contributing factors to social behavior in rodent models of autism spectrum disorder. Dev. Neurobiol. 78, 474-499 (2018).

Needham, B.D., Adame, M.D., Serena, G., Rose, D.R., Preston, G.M., Conrad, M.C., Campbell, A.S., Donabedian, D.H., Fasano, A., Ashwood, P., and Mazmanian, S.K.: Plasma and Fecal Metabolite Profiles in Autism Spectrum Disorder. Biol. Psychiatry 89, 451-462 (2021).

Needham, B.D., Funabashi, M., Adame, M.D., Wang, Z., Boktor, J.C., Haney, J., Wu, W.L., Rabut, C., Ladinsky, M.S., Hwang, S.J., Guo, Y., Zhu, Q., Griffiths, J.A., Knight, R., Bjorkman, P.J., Shapiro, M.G., Geschwind, D.H., Holschneider, D.P., Fischbach, M.A., and Mazmanian, S.K.: A gut-derived metabolite alters brain activity and anxiety behaviour in mice. Nature 602, 647-653 (2022).

Neufeld, K.M., Kang, N., Bienenstock, J., and Foster, J.A.: Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol. Motil. 23, 255-264 (2011).

Neuman, H., Forsythe, P., Uzan, A., Avni, O., and Koren, O.: Antibiotics in early life: dysbiosis and the damage done. FEMS Microbiol. Rev. 42, 489-499 (2018).

Niesler, B. and Rappold, G.A.: Emerging evidence for gene mutations driving both brain and gut dysfunction in autism spectrum disorder. Mol. Psychiatry 26, 1442-1444 (2021).

Otten, K., Keller, L., Puiu, A.A., Herpertz-Dahlmann, B., Seitz, J., Kohn, N., Edgar, J.C., Wagels, L., and Konrad, K.: Pre- and postnatal antibiotic exposure and risk of developing attention deficit hyperactivity disorder-A systematic review and meta-analysis combining evidence from human and animal studies. Neurosci. Biobehav. Rev. 140, 104776 (2022).

Ozonoff, S., Young, G.S., Belding, A., Hill, M., Hill, A., Hutman, T., Johnson, S., Miller, M., Rogers, S.J., Schwichtenberg, A.J., Steinfeld, M., and Losif, A.-M.: The broader autism phenotype in infancy: when does it emerge? J. Am. Acad. Child Adolesc. Psychiatry 53, 398-407 (2014).

Palmeira, O., Matos, L.R.B., Naslavsky, M.S., Bueno, H.M.S., Soler, J.P., Setubal, J.C., and Zatz, M.: Longitudinal 16S rRNA gut microbiota data of infant triplets show partial susceptibility to host genetics. iScience 25, 103861 (2022).

Pusceddu, M.M., Barboza, M., Keogh, C.E., Schneider, M., Stokes, P., Sladek, J.A., Kim, H.J.D., Torres-Fuentes, C., Goldfild, L.R., Gillis, S.E., Brust-Mascher, I., Rabasa, G., Wong, K.A., Lebrilla, C., Byndloss, M.X., Maisonneuve, C., Bäumler, A.J., Philpott, D.J., Ferrero, R.L., Barrett, K.E., Reardon, C., and Gareau, M.G.: Nod-like receptors are critical for gut-brain axis signalling in mice. J. Physiol. 597, 5777-5797 (2019).

Royet, J., Gupta, D., and Dziarski, R.: Peptidoglycan recognition proteins: modulators of the microbiome and inflammation.

Nat. Rev. Immunol. 11, 837-851 (2011).
Sandler, R.H., Finegold, S.M., Bolte, E.R., Buchanan, C.P., Maxwell, A.P., Vaisanen, M.L., Nelson, M.N., and Wexler, H.M.: Short-term benefit from oral vancomycin treatment of regressive-onset autism. J. Child. Neurol. 15, 429-435 (2000).
Sgritta, M., Dooling, S.W., Buffington, S.A., Momin, E.N., Francis, M.B., Britton, R.A., and Costa-Mattioli, M.: Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. Neuron 101, 246-259 (2019).
Sharon, G., Cruz, N.J., Kang, D.W., Gandal, M.J., Wang, B., Kim, Y.M., Zink, E.M., Casey, C.P., Taylor, B.C., Lane, C.J., Bramer, L.M., Isern, N.G., Hoyt, D.W., Noecker, C., Sweredoski, M.J., Moradian, A., Borenstein, E., Jansson, J.K., Knight, R., Metz, T.O., Lois, C., Geschwind, D.H., Krajmalnik-Brown, R., and Mazmanian, S.K.: Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell 177, 1600-1618 (2019).
Swann, J.R., Spitzer, S.O., and Diaz Heijtz, R.: Developmental Signatures of Microbiota-Derived Metabolites in the Mouse Brain. Metabolites 10, 172 (2020).
Tabouy, L., Getselter, D., Ziv, O., Karpuj, M., Tabouy, T., Lukic, I., Maayouf, R., Werbner, N., Ben-Amram, H., Nuriel-Ohayon, M., Koren, O., and Elliot, E.: Dysbiosis of microbiome and probiotic treatment in a genetic model of autism spectrum disorders. Brain Behav. Immun. 73, 310-319 (2018).
Thion, M.S., Low, D., Silvin, A., Chen, J., Grisel, P., Schulte-Schrepping, J., Blecher, R., Ulas, T., Squarzoni, P., Hoeffel, G., Coulpier, F., Siopi, E., David, F.S., Scholz, C., Shihui, F., Lum, J., Amoyo, A.A., Larbi, A., Poidinger, M., Buttgereit, A., Lledo, P.-M., Greter, M., Chan, J.K.Y., Amit, I., Beyer, M., Schultze, J.L., Schlitzer, A., Pettersson, S., Ginhoux, F., and Garel, S.: Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific Manner. Cell 172, 500-516 (2018).
Vuong, H.E., Pronovost, G.N., Williams, D.W., Coley, E.J.L., Siegler, E.L., Qiu, A., Kazantsev, M., Wilson, C.J., Rendon, T., and Hsiao, E.Y.: The maternal microbiome modulates fetal neurodevelopment in mice. Nature 586, 281-286 (2020).
Wang, L.W., Tancredi, D.J., and Thomas, D.W.: The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected members. J. Dev. Behav. Pediatr. 32, 351-360 (2011).
Wheeler, R., Bastos, P.A.D., Disson, O., Rifflet, A., Gabanyi, I., Spielbauer, J., Berard, M., Lecuit, M., and Boneca, I.G.: Microbiota-induced active translocation of peptidoglycan across the intestinal barrier dictates its within-host dissemination. Proc. Natl. Acad. Sci. USA 120, e2209936120 (2023).
Xu, M., Xu, X., Li, J., and Li, F.: Association Between Gut Microbiota and Autism Spectrum Disorder: A Systematic Review and Meta-Analysis. Front. Psychiatry 10, 473 (2019).
Yap, C.X., Henders, A.K., Alvares, G.A., Wood, D.L.A., Krause, L., Tyson, G.W., Restuadi, R., Wallace, L., McLaren, T., Hansell, N.K., Cleary, D., Grove, R., Hafekost, C., Harun, A., Holdsworth, H., Jellett, R., Khan, F., Lawson, L.P., Leslie, J., Frenk, M.L., Masi, A., Mathew, N.E., Muniandy, M., Nothard, M., Miller, J.L., Nunn, L., Holtmann, G., Strike, L.T., de Zubicaray, G.I., Thompson, P.M., McMahon, K.L., Wright, M.J., Visscher, P.M., Dawson, P.A., Dissanayake, C., Eapen, V., Heussler, H.S., McRae, A.F., Whitehouse, A.J.O., Wray, N.R., and Gratten, J.: Autism-related dietary preferences mediate autism-gut microbiome associations. Cell 184, 5916-5931 (2021).
Zeidan, J., Fombonne, E., Scorah, J., Ibrahim, A., Durkin, M.S., Saxena, S., Yusuf, A., Shih, A., and Elsabbagh, M.: Global prevalence of autism: A systematic review update. Autism Res. 15, 778-790 (2022).
Zhao, H., Mao, X., Zhu, C., Zou, X., Peng, F., Yang, W., Li, B., Li, G., Ge, T., and Cui, R.:

GABAergic System Dysfunction in Autism Spectrum Disorders. Front. Cell Dev. Biol. 9, 781327 (2021).

Zheng, Y., Bek, M.K., Prince, N.Z., Peralta Marzal, L.N., Garssen, J., Perez Pardo, P., and Kraneveld, A.D.: The Role of Bacterial-Derived Aromatic Amino Acids Metabolites Relevant in Autism Spectrum Disorders: A Comprehensive Review. Front. Neurosci. 15, 738220 (2021).

Zuffa, S., Schimmel, P., Gonzalez-Santana, A., Belzer, C., Knol, J., Bolte, S., Falck-Ytter, T., Forssberg, H., Swann, J., and Diaz Heijtz, R.: Early-life differences in the gut microbiota composition and functionality of infants at elevated likelihood of developing autism spectrum disorder. Transl. Psychiatry 13, 257 (2023).