Old Herborn University Seminar Monograph 26:
The gut microbiome and the nervous system.
Editors: Peter J. Heidt, John Bienenstock, and Volker Rusch.
Old Herborn University Foundation, Herborn-Dill, Germany: 49-59 (2013).

MAPPING THE CONSEQUENCES OF METABOLIC INTERACTIONS BETWEEN HOST AND MICROBIOME ON THE BRAIN

ELAINE HOLMES

Biomolecular Medicine, Imperial College London,
Sir Alexander Fleming Building,
South Kensington, London, SW7 2AZ, UK

INTRODUCTION

The gut microbiome, defined by Joshua Lederberg as the sum of the bacterial genomes and their environmental interactions (*Lederberg,* 2001), acts as an extra organ in the mammalian host with a vast capacity for metabolism of chemicals deriving from endogenous mammalian metabolism, nutrients and other xenobiotics. This surrogate organ not only functions to shape the immune system and harvest energy, but is also capable of direct chemical communication and contributes to a wide range of signalling pathways that connect various organs and tissues throughout the body (*Cryan* and *O'Mahony,* 2011; *Nicholson* et al., 2012). The communication between the gut and the brain, the so called 'gut-brain axis' is a critical factor in many physiological and pathological processes. In addition to direct interaction between the brain and enteric nervous system in the gut via the vagus nerve, a great deal of chemical communication occurs and many of the chemicals produced by the gut microbiota are potentially neuroactive including γ-amino butyric acid (GABA), 5-hydroxytryptophan and indoxyl compounds (*Wikoff* et al., 2009; *Barrett* et al., 2012). Conversely the brain can modulate the behaviour of the microbiota via mechanisms such as stimulation of the endochromaffin cells. This review addresses the influence of the gut microbiota on the brain and central nervous system (CNS) and explores their consequent impact on metabolism under various physiological and pathological conditions.

METABOLIC SIGNATURE OF AUTISM: CONTRIBUTIONS FROM THE MICROBIOME

Autism spectrum disorder (ASD) represents a group of developmental conditions characterised by dysfunction in verbal and non-verbal communication and social interactions and is associated with repetitive behaviour and difficulty in imaginative play in children. First described by Kanner in 1943, autism typically manifests during the first three years of life and is part of the Pervasive Developmental Disorders (PDD) family. It is diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and there are several clinical scoring scales used for establishing the severity and type of autism (*Faras* et al., 2010). A recent review reports that whilst the conservative estimate of autistic spectrum disorder prevalence is 27.5 per 10,000 individuals, the real prevalence estimate based on newer surveys is 60 per 10,000 individuals, with a 4:1 male-to-female ratio (*Faras* et al., 2010), thus autism is a growing clinical, social and financial concern.

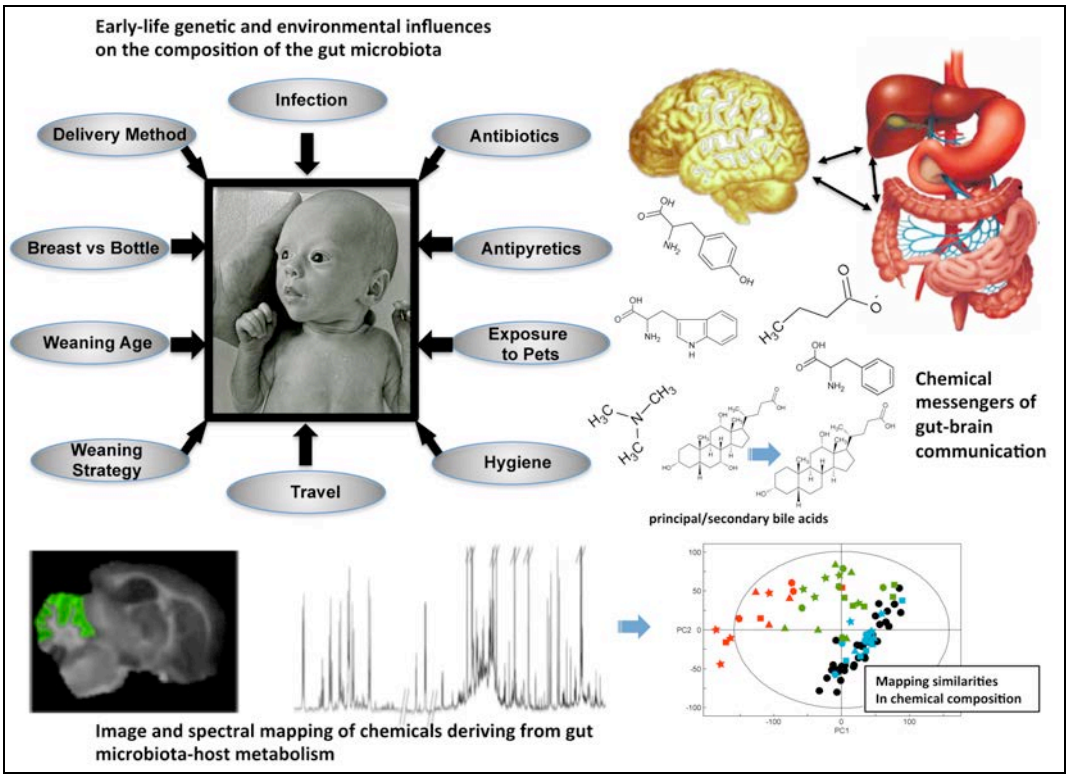

Figure 1: The early life environment influences the composition of the gut microbiota with consequent risk or protection for later-life disease. One of the ways in which the brain and gut are inextricably linked is through chemical dialogue between the gut microbiota and the brain in the form of small molecule messengers such as bile acids, biogenic amines, products of choline degradation and short chain fatty acids. These molecules can be detected in the NMR and MS profiles of urine, plasma, cerebrospinal fluid and other biofluids. The distribution of these chemicals can also be mapped in the brain in order to ascertain their topographical distribution. Computational modelling can be used to efficiently extract and visualise information relating to gut-brain communication and to unravel the profound dependence of humans on their microbiota.

Clinicians, scientists and parents of autistic children have proposed multiple theories regarding the causes of autism. However, only limited clinical data are available and in some cases the data are contradictory and/or inconclusive. Numerous aetiological hypotheses have been postulated including: *In utero* exposure to chemicals such as pesticides, genetic causes (particular association with the X chromosome), oxidative stress, measles, mumps and rubella (MMR) vaccination or its thiomersal carrier, advanced maternal age, planned caesarean section, low Apgar scores, hyperbilirubinaemia and birth defects (*Duchan* et al., 2012; *Guinchat* et al., 2012). Inflammation has also been associated with autism and could be mediated by a defective placenta, immature blood-brain barrier, the immune response of the mother to infection while pregnant, a premature birth, encephalitis in the child after birth, or a toxic environment either *in utero* or in the first few months of life (*Faras* et al., 2010). Although some of the theories on the causes of autism, such as the MMR vaccine, have been largely discredited, it is evident that ASD repre-

sents a complex set of interrelated conditions and is likely to originate from a highly multifactorial aetiology. This is further supported by the vast array of co-morbidities that typically accompany autism, in particular gastrointestinal dysfunction. Understanding the role of the gut microbiota in autism would undoubtedly offer new therapeutic avenues, regardless of whether the intestinal dysfunction is causal or merely coincidental.

The compositional landscape and functionality of the gut microbiota are determined by a sequence of factors beginning at or before birth. The first large-scale exposure to bacteria occurs during the birth process and continues to be modulated by subsequent exposure to the external environment, feeding method and early life infections. Whilst the early microbiotal composition is dynamic, this plasticity diminishes over time to form a relatively stable microbiome that persists throughout adulthood. However, it is thought that these early influences on the microbiota can persist and influence disease risk later in life. For example preterm infants have a higher risk of cardiovascular disease, pulmonary disease, metabolic diseases and several psychological and neurodevelopmental conditions including autism (*Rogers* and *Velten*, 2011). Metabolic profiling of biofluids and tissues can detect changes in microbial activity via profiling or imaging of microbial products such as cresols, biogenic amines, short chain fatty acids etc. (Figure 1), and represents one means of understanding the relationship between the gut and the brain.

Gastrointestinal (GI) dysfunction is reported in a high percentage of children diagnosed with autism with reported symptoms ranging from diarrhoea to constipation and abdominal bloating and pain. In one study, intestinal permeability was found to be higher in individuals with ASD (36.7%) in comparison with healthy controls (4.8%), whereas ASD participants on a casein free diet did not show this increase in intestinal permeability (*de Magistris* et al., 2012). Interestingly, family members of autistic children also showed increased intestinal permeability. Given the prevalence of GI dysfunction in children with ASD, there has been growing interest in the role of the gut microbiota, specifically bacterial populations such as the Clostridia, which are known to produce toxins and often flourish in a perturbed gut ecosystem. However, as yet the literature contains few examples of studies in which the microbiome of autistic individuals has been systematically characterised and those that have been reported suffer from a low sample size and poor comparison between autistic children with and without GI dysfunction (*Critchfield* et al., 2011). Williams and colleagues showed in a study of the mucosal biopsies of 22 children with autism that disaccharidases and hexose transporters were deficient and that this deficiency correlated with the intestinal transcription factor caudal type homeobox 2 (CDX2) that regulates transporters of glucose and other sugars (*Williams* et al., 2011). This change in transcription factors was correlated with the bacterial profiles of the mucosal samples that showed an increase in the *Firmicute:Bacteroidete* ratio and increased numbers of Betaproteobacteria.

A series of relatively small scale studies have demonstrated that autistic individuals harbour a distinctive faecal microflora compared with that of healthy children (*Parracho* et al., 2005; *Finegold* et al., 2010), with autistic children carrying greater numbers of bacteria of the *Clostridium histolyticum* group (*Parracho* et al., 2005) and *Desulfovibrio* (*Finegold* et al., 2010)

and manifesting a shift at the phylum level towards an increased *Bacteroidetes:Firmicute* ratio (*Finegold* et al., 2010). Other bacteria found in high concentrations in the epithelial mucosa of children with ASD associated GI symptoms are the *Sutterella* genus, predominantly *Sutterella wadsworthensis* and *Sutterella stercoricanis* (*Williams* et al., 2012).

Metabolic profiling studies have also generated indirect evidence of a shift in clostridial species. Urinary excretion of 4-cresyl sulphate is increased in autistic children, and to a lesser extent their siblings (*Yap* et al., 2010). 4-Cresol is a gut microbial metabolite that can be synthesized by several bacteria including *Clostridium difficile* and *Clostridium scatalogens*, and which subsequently undergoes phase II metabolism to the sulphate conjugate with minor amounts of the glucuronide conjugate formed. Children with ASD have also been found to have higher urinary levels of another clostridial metabolite, 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), which is thought to be a metabolite of the tyrosine analogue 3-hydroxyphenylalanine (*Shaw,* 2010). This metabolite was also found in high levels in the urine of an adult that had experienced repeated *C. difficile* infections (*Shaw,* 2010).

Other metabolites that characterize the urine of autistic children include bacterial products of choline degradation such as methylamine and dimethylamine, hippurate, phenylacetylglutamine, reinforcing the possible involvement of the microbiota in autism. In contrast, Lis and colleagues. reported lower urinary levels of hippurate and 4-hydroxyhippurate in autistic children compared to age matched controls, as measured by ion exchange chromatography (*Lis* et al., 1976). Thus the relationship between autism and the gut microbiota is not straightforward, like most other aspects of ASD.

As with the role of the gut microbiota, the involvement of oxidative stress in the aetiology of ASD is similarly ambiguous. Both oxidized glutathione and the ratio of oxidized to reduced glutathione are increased in autism (*Ghanizadeh* et al., 2012; *Frustaci* et al., 2012), whilst blood levels of reduced glutathione, glutathione peroxidase and sulphur-containing amino acids cysteine and methionine were found to be reduced in a meta analysis of ASD (*Frustaci* et al., 2012). There is some evidence that dietary supplementation with glutathione may improve the levels of trans-sulphuration metabolites (*Kern* et al., 2011).

Low levels of reduced plasma glutathione and sulphur-containing amino acids are consistent with the observation that autistic children are deficient in sulphation capacity (*Alberti* et al., 1999), thus any intervention that challenges the already impaired capacity for sulphation has the potential to compound the expression of ASD. Acetaminophen use in children has been suggested as a trigger for autism via activation of the endocannabinoid system, which is known to be capable of modulating brain function during development (*Schultz*, 2010). The primary route of acetaminophen excretion in children is sulphation. In autistic children that are already sulphur deficient, acetaminophen could further deplete already low sulphur pools with subsequent impact on the glutathione pathway. Interestingly the metabolic fate of acetaminophen can be predicted from pre-dose urinary profiles and the most predictive metabolite for high acetaminophen sulphate excretion is 4-cresyl sulphate (*Clayton* et al., 2009), the synthesis of which has been largely attributed to *Clostridium difficile* and *C. scatalogens*. Thus it is possible that the gut microbiota contribute to the deple-

tion of sulphate pools in autistic children by generating phenols and other chemicals that undergo sulphation prior to excretion.

In conclusion, the role of the gut microbiota in ASD is far from clear. Various studies show differential microbial profiles between autistic and healthy children, and the presence of various GI-related co-morbidities points to an association between the microbiome and ASD. The fact that children with ASD tend towards food sensitivity and are typically on numerous therapeutic regimes including controlled diets, chelation agents and a cocktail of drugs such as ariprazole, altrexone, buspirone, divalproex sodium, lamotrigine, levetiracetam, memantine, mirtazapine, riluzole, pioglitazone, and topiramate (*Doyle and McDougle*, 2012) confounds the picture as these agents may in themselves induce a change in the gut microbiota. This underscores the requirement for high quality, systematic and controlled studies to be carried out in order to ascertain the true relationship between the gut microbiota and ASD.

INFECTION-INDUCED DISRUPTION OF THE GUT MICROBIOTA-HOST METABOLIC INTERFACE

Both chronic and acute infections have been shown to have profound impact on the gut microbiome. Reduced cognitive function in children in developing countries has been correlated with repeated incidences of infection causing diarrhoea (*Oria* et al., 2009). Evidence of both direct and indirect influence of the microbiota on the central nervous system (CNS) can be found in the literature. For example, increased anxiety, brought about through viscerosensory signalling from the gastrointestinal tract, can be induced in mice by experimental infection with *Campylobacter jejuni* (*Goehler* et al., 2007).

The hygiene hypothesis has lead to the association of certain autoimmune diseases with infection and bacteria. The onset of these diseases has been linked to bacterially driven immune responses. In an animal model of multiple sclerosis, it has been shown that commensal gut microbiota are necessary for initiating immune responses that result in myelin-specific CD4(+) T cells attacking the CNS (*Berer* et al., 2011).

Examples in both the insect and in the human world provide evidence that parasitic infection can alter behaviour. Parasitic hairworms manipulate their cricket host by inducing suicide behaviour, causing the insect to leap into water, where the parasite continues its life cycle (*Ponton* et al., 2011). Epidemiological studies have found associations between certain helminths, including *Schistosoma mansoni, Ascaris lumbracoides* and *Trichuris trichiura,* and cognitive impairment in school age children (*Jardim-Botelho* et al., 2008; *Shang* and *Tang*, 2010) but the mechanism is unknown. Direct contact of a parasite with the CNS can result in overt histological and biochemical changes. Most prominent examples are cerebral manifestations of malaria, due to malaria toxin excretion, excess cytokine secretion and billharziosis-induced granuloma formation caused by sequestration of Schistosome erythrocytes. Serendipitous ectopic worm manifestations in the brain by cestodes, trematodes and protozoa have also been reported to cause lesions and haemorrhages (*Walker* et al., 2005). However, for most host-parasite interactions involving neurological damage or altered behaviour, the molecular mechanisms

are not well understood.

There is some suggestion that certain changes in brain biochemistry following helminth infection may be associated with dysregulation of the microbiota. The parasitology literature contains several examples of infection-induced disturbances of the microbiota (*Wang* et al., 2010; *Li* et al., 2012). Many parasites ranging from *Giardia* to hookworm are associated with gastrointestinal disturbances. It is unsurprising that parasites that reside in the gut should impact upon the microbiota, since they share the same physical environment. However, studies in animal models have shown that several parasites, some of which reside in the blood or in non-gastrointestinal tissues, can modulate the excretion of gut microbial metabolites.

Parasite infections have been shown to perturb several metabolites of the gut microbiota in various animal models and in humans (*Wang* et al., 2004, 2010). Metabolic profiling studies in humans and animal models have shown modulation of urinary gut microbial metabolites including hippurate, 4-cresyl sulphate and glucuronide, phenylacetyl glycine / glutamine, 4-hydroxyphenylacetic acid and 4-hydroxy-3-methy-phenylpropionate acid (*Wang* et al, 2004, *Wang* et al, 2010; *Balog* et al, 2011). In a study investigating the metabolic effects of a foodborne trematode, *Fasciola hepatica*, in a rat model, clear evidence of altered gut microbial metabolism was found in both the urine (decreased hippurate levels and modified bacterial products of choline degradation) and faecal metabolite profiles and, more interestingly, these changes correlated with altered brain biochemistry following infection. Significant perturbations of the nucleotide balance in the brain suggested a shift toward modulation of immune reactions, which could serve to prolong the camouflage of the parasite within the host via minimization of inflammatory damage (*Saric* et al., 2010).

Thus, it seems that there is a complex tripartite interaction between host, parasite and microbiota mediated by both immunoregulatory mechanisms and direct chemical communication between microbe and host. Moreover, this three-way relationship has system-wide impact, including neurological consequences and warrants deeper interrogation.

METABOLIC EFFECTS OF THE GUT MICROBIOTA IN RELATION TO IMPACT ON MOOD AND BEHAVIOUR

There is a growing body of evidence associating depression and other psychiatric disorders with perturbed gut microbiota. The cytokine hypothesis of depression suggests that increased translocation of LPS from Gram-negative bacteria and inflammation are causally associated with clinical depression and neurodegeneration (*Qin* et al., 2007; *Maes*, 2008). In another stress-induced rat model of depression associated with leaky gut, the administration of *Lactobacillus farciminis* was shown to reverse the stress-induced hyperpermeability and behavioural changes (*Ait-Belgnaoui* et al., 2012) In addition to producing LPS and other neurotoxic chemicals, the gut bacteria can synthesize metabolites such as pyrogallol and urolithins from dietary polyphenols that are neuroprotective and can counteract the effects of diabetes-induced neurodegeneration (*Verzelloni* et al., 2011).

Gut microbial metabolites found in urine and other biofluids in animal and

human studies of depression also point to the involvement of the gut bacteria in behaviour. An LC-MS profiling study of a rat model of depression found elevated urinary levels of kynurenic acid, hippurate, phenylacetylglycine and xanthurenic acid with lower levels of tryptophan, indoxyl sulphate, indole-3-acetate and tricarboxylic acid cycle intermediates in rats challenged by chronic unpredictable mild stress suggesting that gut microbial metabolism and modulation of the TCA cycle were key components of this model of depression (*Zheng* et al., 2009). Interferon-α administration has been associated with depressive disorders in cancer or hepatitis-C patients treated with interferon-α (*Raison* et al., 2006). Interferon-α induced activation of the tryptophan degrading enzyme indoleamine 2,3-dioxygenase results in the generation of quinolinic acid and other neuroactive metabolites, which are thought to relate to depression. In a bacterially-induced animal model of depression, induced by inoculation with Bacillus Calmette-Guérin (BCG), administration of indoleamine 2,3-dioxygenase resulted in increased plasma concentrations of the kynurenine:tryptophan ratio (*O'Connor* et al., 2009).

Examples of the effect of microbiota on cognition are scattered throughout the literature. As previously mentioned, repeated diarrhoeal infections have been associated with detrimental effects on cognition in children. Similarly cirrhotic patients with encephalopathy show poor cognition, which is correlated with *Veillonellaceae* in faecal material (*Bajaj* et al., 2012). Higher faecal levels of *Enterobacteriaceae, Alcaligeneceae* and *Fusobacteriaceae* with lower levels of *Ruminococcaceae* and *Lachnospiraceae* were also found in cirrhotic patients.

There is increasing acceptance of the fact that surgery *per se* can impose short-term or long-term consequences on mood, behaviour and cognition. Several analyses of patients undergoing coronary artery bypass graft surgery (CABG) have indicated that neurological complications can include stroke, depression or mild cognitive decline reflected by short-term memory loss or psychomotor slowing (*Hawkes* et al., 2006). The cause of these neurological effects is not well understood but surgical trauma, genetic susceptibility, interoperative or post-operative ischaemia and body temperature during surgery have all been proposed as causal factors (*Hawkes* et al., 2006). Probiotics and synbiotics (a combination of pre- and probiotics) have been used to reduce the incidence of post-operative sepsis after elective surgery and to counteract the effect of antibiotics, typically administered as part of surgical procedures (*Kinross* et al., 2012). Prebiotics have also been shown to influence anxiety and mood. For example, Silk and colleagues showed that administration of a trans-galacto-oligosaccharide enhanced faecal bifidobacteria and improved anxiety and depression symptoms in patients with irritable bowel syndrome (*Silk* et al., 2009). Similarly probiotics such as lactobacilli have been reported to regulate emotional behaviour in mice through vagus nerve stimulated modulation of GABA receptor expression (*Bravo* et al., 2011).

Obesity has been associated with poor cognitive function, particularly in executive function (*Lokken* et al., 2010). There is controversy regarding the effect of weight loss surgery (bariatric surgery) on cognition and memory with some suggestion that bariatric surgery can cause memory impairment and depression. An animal model of gastric restriction was able to show structural alterations in the hippocampus (*Sonoda* et al., 2011). The mecha-

nisms of bariatric surgery-induced weight loss are known to involve alteration of gastrointestinal hormones, which are important in appetite regulation. Several gastrointestinal hormones can contribute to obesity by modulating the activity of the gut-brain axis. Recently we have characterized the metabolic response of rats undergoing RYGB surgery using high resolution spectroscopic profiling and integrated the response with the post surgical change in the microbiome and shown a dramatic shift towards the gammaproteobacteria. The surgery also resulted in a modulation of gut microbial metabolites, some of which were neuroactive. Increased urinary cresols, phenylacetylglycine and indoxylsulfate occurred post surgery together with increased production of γ-aminobutyric acid (GABA) (*Li* et al., 2011).

CONCLUDING REMARKS

The manner in which the gut bacteria influence the CNS largely remains elusive and merits systematic study. The gut and brain use several communication axes involving nerve stimulation, immunological correspondence and direct metabolic interactions in order to maintain homeostasis. The gut microbiota contribute to the communication highway between the gut and the brain and there is enough evidence to implicate the microbiota in various neurodegenerative diseases, behavioural disorders, neurodevelopmental conditions and cognition. The partnership between culture independent sequencing of the microbiome, the human genome and metabolic profiling of the microbial components of biofluids should help to elucidate the complex interactions of host and microbe and to place this interaction in terms of regulation/ dysregulation of the CNS. Understanding this partnership will open new avenues for therapeutic intervention and opportunities for improving disease risk by modulation of the microbiota early in life or even prenatally.

LITERATURE

Alberti, A., Pirrone, P., Elia, M., Waring, R.H., and Romano, C.: Sulphation deficit in "low-functioning" autistic children: A pilot study. Biol. Psychiatry 46, 420-424 (1999).

Ait-Belgnaoui, A., Durand, H., Cartier, C., Chaumaz, G., Eutamene, H., Ferrier, L., Houdeau, E., Fioramonti, J., Bueno, L., and Theodorou, V.: Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology 37, 1885-1895 (2012).

Bajaj, J.S., Ridlon, J.M., Hylemon, P.B., Thacker, L.R., Heuman, D.M., Smith, S., Sikaroodi, M., and Gillevet, P.M.: Linkage of gut microbiome with cognition in hepatic encephalopathy. Am. J. Physiol. Gastrointest. Liver Physiol. 302, G168-G175 (2012).

Balog, C.I., Meissner, A., Goraler, S., Bladergroen, M.R., Vennervald, B.J., Mayboroda, O.A., and Deelder, A.M.: Metabonomic investigation of human *Schistosoma mansoni* infection. Mol. Biosyst. 7, 1473-1480 (2011).

Barrett, E., Ross, R.P., O'Toole, P.W., Fitzgerald, G.F., and Stanton, C.: Gamma-aminobutyric acid production by culturable bacteria from the human intestine. J. Appl. Microbiol. 113, 411-417 (2012).

Berer, K., Mues, M., Koutrolos, M., Rasbi,

Z.A., Boziki, M., Johner, C., Wekerle, H., and Krishnamoorthy, G.: Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature 479, 538-541 (2011).

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).

Clayton, T.A., Baker, D., Lindon, J.C., Everett, J.R., and Nicholson, J.K.: Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. Proc. Natl. Acad. Sci. USA 106, 14728-14733 (2009).

Critchfield, J.W., van Hemert, S., Ash, M., Mulder, L., and Ashwood, P.: The potential role of probiotics in the management of childhood autism spectrum disorders. Gastroenterol. Res. Pract. 2011:161358, Epub (2011).

Cryan, J.F. and O'Mahony, S.M.: The microbiome-gut-brain axis: From bowel to behavior. Neurogastroenterol. Motil. 23, 187-192 (2011).

da Mota, F.F., Marinho, L.P., Moreira, C.J., Lima, M.M., Mello, C.B., Garcia, E.S., Carels, N., and Azambuja, P.: Cultivation-independent methods reveal differences among bacterial gut microbiota in triatomine vectors of Chagas disease. PLoS Negl. Trop. Dis. 6, e1631 (2012).

de Magistris, L., Familiari, V., Pascotto, A., Sapone, A., Frolli, A., Iardino, P., Carteni, M., De Rosa, M., Francavilla, R., Riegler, G., Militerni, R., and Bravaccio, C.: Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J. Pediatr. Gastroenterol. Nutr. 51, 418-424 (2010).

Doyle, C.A. and McDougle, C.J.: Pharmacotherapy to control behavioral symptoms in children with autism. Expert. Opin. Pharmacother. 13, 1615-1629 (2012).

Duchan, E. and Patel, D.R.: Epidemiology of autism spectrum disorders. Pediatr. Clin. North Am. 59, 27-43 (2012).

Faras, H., Al Ateeqi, N., and Tidmarsh, L.: Autism spectrum disorders. Ann. Saudi Med. 30, 295-300 (2010).

Finegold, S.M., Dowd, S.E., Gontcharova, V., Liu, C., Henley, K.E., Wolcott, R.D., Youn, E., Summanen, P.H., Granpeesheh, D., Dixon, D., Liu, M., Molitoris, D.R., and Green, J.A. 3rd.: Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 16, 444–453 (2010).

Frustaci, A., Neri, M., Cesario, A., Adams, J.B., Domenici, E., Dalla Bernardina, B., and Bonassi, S.: Oxidative stress-related biomarkers in autism: Systematic review and meta-analyses. Free Radic. Biol. Med. 52, 2128-2141 (2012).

Ghanizadeh, A., Akhondzadeh, S., Hormozi, M.A, Abotorabi, M., and Firoozabadi, A.: Glutathione-related factors and oxidative stress in autism, a review. Curr. Med. Chem. 19, 4000-4005 (2012).

Goehler, L.E., Lyte, M., and Gaykema, R.P.: Infection-induced viscerosensory signals from the gut enhance anxiety: implications for psychoneuroimmunology. Brain Behav. Immun. 21, 721-726 (2007).

Guinchat, V., Thorsen, P., Laurent, C., Cans, C., Bodeau, N., and Cohen, D.: Pre-, peri- and neonatal risk factors for autism. Acta Obstet. Gynecol. Scand. 91, 287-300 (2012).

Hawkes, A.L., Nowak, M., Bidstrup, B., and Speare, R.: Outcomes of coronary artery bypass graft surgery. Vasc. Health Risk Manag. 2, 477-484 (2006).

Jardim-Botelho, A., Raff, S., Rodrigues, R.A, Hoffman, H.J., Diemert, D.J., Correa-Oliveira, R., Bethony, J.M., and Gazzinelli, M.F.: Hookworm, *Ascaris lumbricoides* infection and polyparasitism associated with poor cognitive performance in Brazilian schoolchildren. Trop. Med. Int. Health 13, 994-1004 (2008).

Kern, J.K., Geier, D.A., Adams, J.B., Garver, C.R., Audhya, T., and Geier, M.R.: A clinical trial of glutathione supplementation in

autism spectrum disorders. Med. Sci. Monit. 17, CR677-682 (2011).

Kinross, J.M., Marakar, S., Karthikesalingam, A., Chow, A., Penney, N., Silk, D., and Darzi, A.: A meta-analysis of probiotic and synbiotic use in elective surgery: Does nutrition modulation of the gut microbiome improve clinical outcome? JPEN J. Parenter. Enteral Nutr. 2012 Jul 10, Epub ahead of print (2012).

Lederberg, J. and McCray, A.T.: 'Ome Sweet 'Omics—a genealogical treasury of words. The Scientist 17, No. 7 (2001).

Li, J.V., Ashrafian, H., Bueter, M., Kinross, J., Sands, C., le Roux, C.W., Bloom, S.R., Darzi, A., Athanasiou, T., Marchesi, J.R., Nicholson, J.K., and Holmes, E.: Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. Gut 60, 1214-1223 (2011).

Li, R.W., Wu, S., Li, W., Navarro, K., Couch, R.D., Hill, D., and Urban, J.F. Jr.: Alterations in the porcine colon microbiota induced by the gastrointestinal nematode *Trichuris suis*. Infect. Immun. 80, 2150-2157 (2012).

Lis, A.W., Mclaughlin, I., Mclaughlin, R.K., Lis, E.W., and Stubbs, E.G.: Profiles of ultraviolet-absorbing components of urine from autistic children, as obtained by high-resolution ion-exchange chromatography. Clin. Chem. 22, 1528-1532 (1976).

Lokken, K.L., Boeka, A.G., Yellumahanthi, K., Wesley, M., and Clements, R.H.: Cognitive performance of morbidly obese patients seeking bariatric surgery. Am. Surg. 76, 55-59 (2010).

Maes, M.: The cytokine hypothesis of depression: Inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Neuro Endocrinol. Lett. 29, 287-291 (2008).

Nicholson, J.K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., and Pettersson, S. Host-gut microbiota metabolic interactions. Science 336,1262-1267 (2012).

O'Connor, J.C., Lawson, M.A., Andre, C., Briley, E.M., Szegedi, S.S., Lestage, J., Castanon, N., Herkenham, M., Dantzer, R., and Kelley, K.W.: Induction of IDO by bacille Calmette-Guérin is responsible for development of murine depressive-like behavior. J. Immunol. 182, 3202-3212 (2009).

Oria, R.B., Costa, C.M., Lima, A.A., Patrick, P.D., and Guerrant, R.L.: Semantic fluency: A sensitive marker for cognitive impairment in children with heavy diarrhea burdens? Med. Hypotheses 73, 682-686 (2009).

Parracho, H.M.R.T., Bingham, M.O., Gibson, G.R., and McCartney, A.L.: Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J. Med. Microbiol. 54, 987-991 (2005).

Ponton, F., Otalora-Luna, F., Lefevre, T., Guerin, P.M., Lebarbenchon, C., Duneau, D., Biron, D.G., and Thomas, F.: Water-seeking behavior in worm-infected crickets and reversibility of parasitic manipulation. Behav. Ecol. 22, 392-400 (2011).

Qin, L., Wu, X., Block, M.L., Liu, Y., Breese, G.R., Hong, J.S., Knapp, D.J., and Crews, F.T.: Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55, 453-462 (2007).

Rogers, L.K. and Velten, M.: Maternal inflammation, growth retardation, and preterm birth: Insights into adult cardiovascular disease. Life Sci. 89, 417-421 (2011).

Saric, J., Li, J.V., Utzinger, J., Wang, Y., Keiser, J., Dirnhofer, S., Beckonert, O., Sharabiani, M.T., Fonville, J.M., Nicholson, J.K., and Holmes, E.: Systems parasitology: Effects of *Fasciola hepatica* on the neurochemical profile in the rat brain. Mol. Syst. Biol. 6, 396 (2010).

Shang, Y. and Tang, L.H.: Intelligence level and characteristics of cognitive structure in school-age children infected with soil-transmitted helminthes. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi 28, 423-426 (2010).

Shaw, W.: Increased urinary excretion of a 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), an abnormal phenylalanine

metabolite of Clostridia spp. in the gastrointestinal tract, in urine samples from patients with autism and schizophrenia. Nutr. Neurosci. 13, 135-143 (2010).

Schultz, S.T.: Can autism be triggered by acetaminophen activation of the endocannabinoid system? Acta Neurobiol. Exp. (Wars) 70, 227-231 (2010).

Silk, D.B., Davis, A., Vulevic, J., Tzortzis, G., and Gibson, G.R.: Clinical trial: The effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. Aliment. Pharmacol. Ther. 29, 508-518 (2009).

Sonoda, E.Y., Gomes da Silva, S., Arida, R.M., Giglio, P.N., Margarido, N.F., Martinez, C.A., Pansani, A.P., Maciel Rde, S., Cavalheiro, E.A., and Scorza, F.A.: Hippocampal plasticity in rats submitted to a gastric restrictive procedure. Nutr. Neurosci. 14, 181-185 (2011).

Verzelloni, E., Pellacani, C., Tagliazucchi, D., Tagliaferri, S., Calani, L., Costa, L.G., Brighenti, F., Borges, G., Crozier, A., Conte, A., and Del Rio, D.: Antiglycative and neuroprotective activity of colon-derived polyphenol catabolites. Mol. Nutr. Food Res. 55, Suppl. 1, S35-S43 (2011).

Walker, M.D. and Zunt J.R.: Neuroparasitic Infections: Cestodes, Trematodes, and Protozoans. Semin. Neurol. 25, 262-277 (2005).

Wang, Y., Holmes, E., Nicholson, J.K., Cloarec, O., Chollet, J., Tanner, M., Singer, B.H., and Utzinger, J.: Metabonomic investigations in mice infected with *Schistosoma mansoni*: An approach for biomarker identification Proc. Natl. Acad. Sci. USA 101, 12676-12681 (2004).

Wang, Y., Li, J.V., Saric, J., Keiser, J., Wu, J., Utzinger, J., and Holmes, E.: Advances in metabolic profiling of experimental nematode and trematode infections. Adv. Parasitol. 73, 373-404 (2010).

Wikoff, W.R., Anfora, A.T., Liu, J., Schultz, P.G., Lesley, S.A., Peters, E.C., and Siuzdak, G.: Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc. Natl. Acad. Sci. USA 106, 3698-3703 (2009).

Williams, B.L., Hornig, M., Buie, T., Bauman, M.L., Cho Paik, M., Wick, I., Bennett, A., Jabado, O., Hirschberg, D.L., and Lipkin, W.I.: Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances. PLoS One. 6, e24585 (2011).

Williams, B.L., Hornig, M., Parekh, T., and Lipkin, W.I.: Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of *Sutterella* species in intestinal biopsy samples from children with autism and gastrointestinal disturbances. MBio. 3, e00261-11 (2012).

Yap, I.K., Angley, M., Veselkov, K.A., Holmes, E., Lindon, J.C., and Nicholson, J.K.: Urinary metabolic phenotyping differentiates children with autism from their unaffected siblings and age-matched controls. J. Proteome Res. 9, 2996-3004 (2010).

Zheng, S., Yu, M., Lu, X., Huo, T., Ge, L., Yang, J., Wu, C., Li, F.: Urinary metabonomic study on biochemical changes in chronic unpredictable mild stress model of depression. Clin. Chim. Acta 411, 204-209 (2010).