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THE MICROBIOME IN AUTISM SPECTRUM DISORDER

The human gut microbiota with reference to autism spectrum disorder: considering the whole as more than a sum of its parts

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The human gut microbiota is a complex microbial ecosystem that contributes an important component towards the health of its host. This highly complex ecosystem has been underestimated in its importance until recently, when a realization of the enormous scope of gut microbiota function has been (and continues to be) revealed. One of the more striking of these discoveries is the finding that the gut microbiota and the brain are connected, and thus there is potential for the microbiota in the gut to influence behavior and mental health. In this short review, we outline the link between brain and gut microbiota and urge the reader to consider the gut microbiota as an ecosystem 'organ' rather than just as a collection of microbes filling a niche, using the hypothesized role of the gut microbiota in autism spectrum disorder to illustrate the concept.

Keywords: *Autism Spectrum Disorder*; *microbiota*; *human*; *gastrointestinal tract*

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In recent years, research into the human microbiome has captured the imagination of the general public, much in the same way that human genome research permeated public consciousness at the start of the new millennium. As a field of study, human microbiome research has exploded in the last decade (Fig. 1), which has led to a new awareness of the importance of these associated microbes to our overall health. This came as somewhat of a shock to those of us who were raised to think of all microbes as 'germs' to be eradicated; instead, we are beginning to see ourselves as microbe managers, tending to the needs of our microbial 'employees' for mutual benefit. This short review discusses how human-associated microbes – particularly those in the gut – affect health, and how the widespread phenomenon of gut microbial 'dysbiosis' could be driving an epidemic of chronic disease, which may include autism spectrum disorder (ASD).

Origins of the human gut microbiota

Until recently, babies were believed to be born sterile and only populated by microbes on exposure to their first post-delivery environments (1). However, the process of microbial colonization may begin before birth, with transfer between mother and baby taking place via the placenta (2), and perhaps influenced by changes in the mother's microbiome during pregnancy (3, 4). Subsequently, the process of vaginal delivery allows for direct transfer of microbes from the birth canal and the perianal area to the baby (5–7). Finally, breastfeeding seems to provide and support specific microbes during the early phases of colonization within the infant gut (8–10). Throughout infancy and early childhood, there are changes in the gut composition that are related to microbial successions, whereby factors such as diet and host immune status appear to confer a 'permanent resident status' for some microbes but not others (8, 11–13). This process of building a gut microbiota is still poorly understood, but it is believed to be of critical importance, because there is increasing evidence that a window of time exists for the gut microbiota to develop (13). Beginning at the time of weaning, the microbiota composition stabilizes and matures (12, 14); from this point, it can be maintained with only minor

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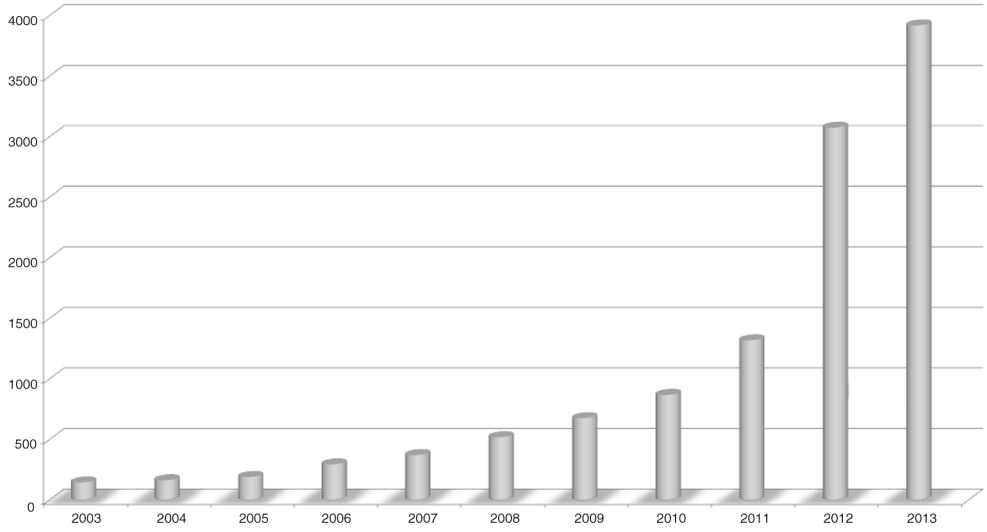

Fig. 1. Trends in human microbiome research over the last decade: PubMed Citations by year using search term 'Human microbiome'. Y-axis: number of publications.

changes over many months or years and perhaps even an entire lifetime (15–18).

Gut microbial ecosystem diversity

The gut microbiota represents one of the densest ecosystems on Earth, and is composed not only of bacteria (which are the most studied components of this niche) but also of Archaea, yeasts, protists, and viruses. Around 500–1,000 different bacterial species may be present in the gut of a given individual (19, 20), although at present the species concept in bacteria is imprecise (because of the propensity for bacteria to share genetic information, for example) (21, 22). The colon is the most densely populated compartment, with bacterial numbers reaching 10^{11}–10^{12} cells per gram of content (23). The viral microbiome (the 'virome') load is estimated to be higher than that of the bacterial load, with the majority being viruses that infect bacteria and Archaea (bacteriophage) (24, 25). Yeasts and other eukaryotes (e.g. protists) are estimated to make up only a small fraction of the colonic microbiota (26, 27).

Diet seems to be an important driver of microbial abundance profiles within an ecosystem (28, 29). Given that most humans are omnivores with diverse diets, this is not surprising; availability of a large selection of dietary substrates promotes the need for a large variety of metabolic pathways for processing, and it is the gut microbiota that takes on the lion's share of this work for its host (30). The resultant microbial diversity, and consequent functional redundancy within an ecosystem, supports overall ecosystem resilience and stability (31, 32). Since resilience and stability largely define the ability of an ecosystem to resist stress, diversity is key to the overall health of the gut microbiota (33).

The functional redundancy of the gut microbiota can also be seen when looking at the human population as a group. There is much variation in the composition of the gut microbiome between individuals, due in large part to the multitude of environmental factors and host genetic influences that work in combination to build a microbiome (30). However, the species variability that can be seen in the microbiomes of different people belies the fact that functionally these microbiomes can be quite similar (34). Even though the exact species content may differ widely, the composite genes of each microbiota as a whole can encode for a very similar group of proteins, or for proteins of similar functions.

Reduced microbial diversity and disease

Having established the importance of microbial community diversity, it is not surprising that a growing body of literature indicates that many chronic diseases are associated with less diverse gut ecosystems (35, 36). At the moment, this phenomenon is mainly associative, as it is difficult to ascertain whether reduced diversity occurs as a result of disease or *vice versa*. However, in some cases (e.g. *Clostridium difficile* infection, CDI), disease certainly results from a loss of gut microbiota diversity and robustness (36). It is undoubtedly true (both on the micro and macro scale) that ecosystems which lack functional redundancy are more prone to collapse under perturbational stress. An imbalance within the microbial ecosystem ('dysbiosis') of the human gut microbiota could result from many different scenarios (Fig. 2), including: insufficient colonization of an infant (e.g. due to Caesarean section) and/or inadequate nursing with breastmilk; exposure to antibiotics, both as short-term therapy as well as long-term pervasive exposure through the food chain; infection with pathogenic microbes; and consumption of a

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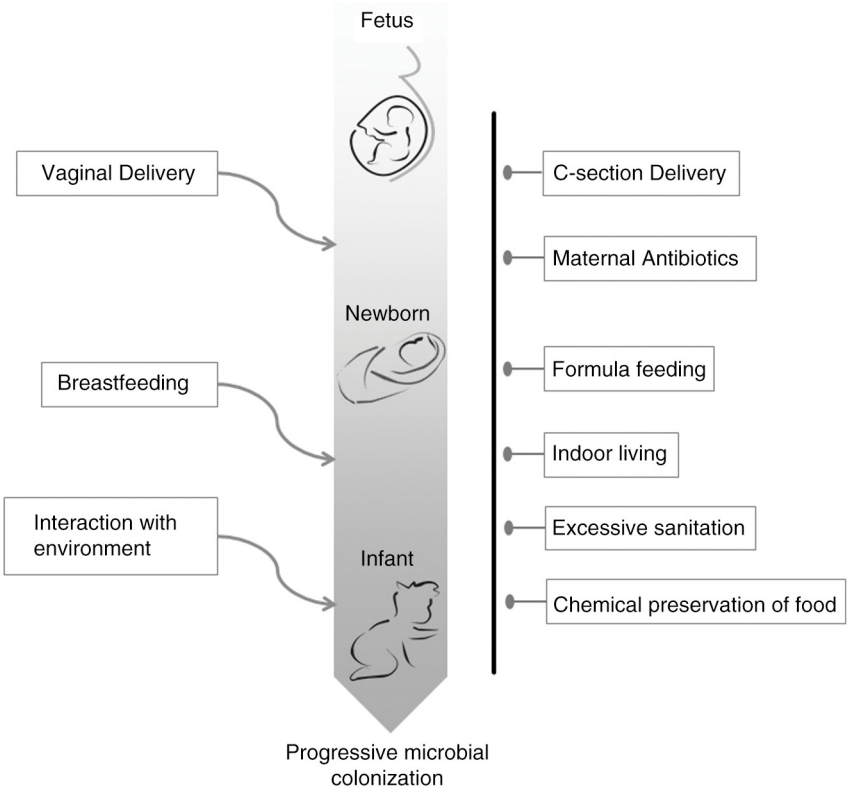

Fig. 2. Pictorial representation of the routes for, and blockages of, microbial colonization of Westernized humans during early life. On the left of the figure, routes of natural colonization are depicted, while on the right, impediments to natural colonization are shown.

refined, Western-style diet with little fiber [which is an important food source for colonic bacteria (29, 37–40)]. Recently, proponents of the 'missing microbiota hypothesis' have warned that modern lifestyles do not sustain a diverse human microbiome, and that the extinction of important 'keystone' microbes could lead to the loss of fundamental functional abilities, which would ultimately contribute to dysbiosis and disease (41).

From a microbiological perspective, the loss of keystone species from the microbiome may impact the subsequent behavior of the remaining microbes. For example, when *C. difficile* is present as part of a diverse gut microbiota, it is unlikely to cause problems to the host because its overall abundance and pathogenic behavior are suppressed by the majority presence of the rest of the microbial species in the ecosystem (36). However, loss of ecosystem diversity, usually brought about by antibiotic use, allows *C. difficile* to proliferate unchecked, and to upregulate virulence determinants that go on to cause disease (42). In this way, *C. difficile* behaves somewhat like a hoodlum in a subway station; when the subway station is crowded with people, the hoodlum is likely to behave appropriately, perceiving scrutiny by the crowd on the platform. But if the subway station is deserted, the hoodlum may start to vandalize the area, his/her behavior influenced by the lack of surveillance. The case of *C. difficile* illustrates the importance of the entire gut microbial ecosystem in disease, rather than individual species. Traditional approaches to clinical microbiology have focused largely on the study and surveillance of specific pathogenic microbial species with well-defined virulence determinants, but our simple 'one microbe-one disease' models will have to change to incorporate a more complete understanding of microbiome dysbiosis in infection.

Microbial conversations

One of the more startling revelations in the field of microbiology is that microbes are able to communicate with each other using chemical languages, with 'words' largely composed of small molecules and peptides (43). Such communication is now known to govern a wide range of functions, including bacterial movement, gene expression, and community structure (44, 45). Furthermore, these same signals may impact the host; small molecules, by their nature, can readily pass through host cells and tissues, and may influence host gene expression and behavior as a result (46, 47). This process could, in fact, be to our evolutionary advantage, forging a beneficial connection between host and symbionts. Laboratory mice that are reared under germ-free conditions can exhibit behavioral and gene expression patterns that differ from those that are raised in conventional conditions (48, 49).

Another striking example of the influence of the gut microbiota on behavior was demonstrated by Bercik et al., who showed that switching the gut microbiota of a timid mouse line (C57Bl/6) with that of a more aggressive mouse line (NIH Swiss) resulted in a concurrent switch in behavioral profiles (50). Bacterial colonization of the gut likely modulates host neural development through signaling pathways that include the use of the vagus nerve, a direct conduit between the gut and the central nervous system (and hence the brain) (51). With the new realization of the influence of the gut microbiota on the brain, is it time that certain diseases traditionally thought to be brain disorders be considered as rooted in the gut microbiota?

The concept of ASD as a consequence of gut microbiota damage

ASD is a pervasive developmental disorder of unknown etiology and widely varying severity. Incidence rates of ASD in North America have risen rapidly in recent decades (52, 53), and although it is important to note that these figures have been influenced by changes in diagnostic practices, heightened public awareness, and varying research methodology, there remains a dramatic upward trend that can only be partly explained by these aforementioned factors. Although ASD is traditionally thought to have strong inheritance, single gene disorders and chromosomal abnormalities only account for a minority of ASD cases (54), and Genome Wide Association Studies have found hundreds of genetic variations to be potentially linked with ASD (55); these observations argue against the prevailing view that the disorder is purely genetic in nature. Many patients on the severe end of the autism spectrum present with gastrointestinal comorbidities that can include diarrhea, constipation, bloating, and abdominal pain (56). The propensity for associated GI issues in many ASD children has led some researchers to hypothesize a gut microbial involvement in disease. Molecular profiling methods have been used to look for differences in the compositions of the gut microbiotas of ASD and healthy individuals by examining stool samples, and different studies have yielded different results. Song et al. found significant increases in *Clostridium bolteae* and *Clostridium* clusters I and XI (57), Finegold et al. noted an increase of *Desulfovibrio* spp. (58), and Wang et al. observed higher levels of *Sutterella* and *Ruminococcus* spp. (59) in individuals with ASD compared to controls. These may not be conflicting findings; instead, these compositional changes could be indicative of gut dysbiosis in ASD, and certainly there is potential here for the development of disease biomarkers. To date, no causative role has been suggested for these, or any other, individual microbial species, and indeed the functional complexity within the gut microbiota argues against a simple one microbe-one disease model.

Given the importance of the functionality of the microbiota over its precise composition, and also taking into account the importance of the gut microbiota in neural development and function, is it possible that a reduction in the gut microbiota diversity leads to a loss of key signals required for normal brain maturation? This could possibly be triggered by the use of antibiotics in early childhood during the critical window for microbiota development, an event that is commonly (if anecdotally) cited by parents of children with ASD; indeed, several studies report increased use of oral antibiotics in children with ASD compared to neurotypical children (60–63). If this is the case, the study of individual components of the gut microbiota will likely not be fruitful as the gut microbial ecosystem as a whole entity must be considered. This is currently limited by technical constraints that we and others are trying to address. Advances in the ability to culture whole gut microbial ecosystems *in vitro* will allow a more holistic view of the structure of the gut microbiota, as well as its potential function in the context of ASD. For example, continuous culture (chemostat) systems allow for the culture of whole, explanted, gut ecosystems under tightly controlled experimental conditions (64), with the added advantage that small molecule metabolites produced by the resident ecosystem can be easily captured and characterized (unpublished observations).

Looking to the future

Is ASD a gut-mediated disease? Clearly there is much work to be done to gain a fuller understanding of what is involved in the etiology of this complex disorder. As with other complex chronic diseases (such as inflammatory bowel disease), ongoing research will likely draw us to the intersection between host genetics and epigenetics, microbiota structure and function, and environmental cues. However, this new and developing view of ASD etiology presents an additional avenue for study.

Conflict of interest and funding

E.A-V is a cofounder of Nubiyota LLC, a company that is engaged in developing and commercializing therapeutic microbial ecosystems for medical use.

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