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RESEARCH HIGHLIGHT

Host genetics, the microbiome & behaviour—a 'Holobiont' perspective

Jatin Nagpal[1] and John F. Cryan[1,2]

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Complex behaviors in genetic neurological disorders have traditionally been viewed through the prism of genetic influences on brain development and function. In a recent study published in *Cell*, Costa-Mattioli and colleagues make a compelling argument that the gut microbiome and the host genes can interdependently regulate different behaviors in a genetic mouse model of autism.

The last two decades have witnessed trailblazing research in the field of neurodevelopmental disorders such as autism spectrum disorder (ASD), unravelling myriad of genes and their effects on developing neural circuits spanning across different brain regions such as cortex, hippocampus, basal ganglia, hypothalamus.[1] These diverse cell types in animal models, primarily rodents, have been examined using advanced molecular and imaging techniques to probe the association between genes' function and behavioral deficits in the animal, focusing on social behavior deficits, repetitive behaviors, anxiety and epilepsy. Harnessing molecular and cellular mechanisms from these preclinical studies, translational studies and clinical trials have gained momentum, though with limited success. Adding to the host genetic variants, a non-genetic yet vertically transmitted determinant that has garnered increasing attention in the aetiology of neurodevelopmental disorders, is the gut microbiota — the complex ecosystem of microbes inhabiting the gastrointestinal tract.

Disruption of gut microbiota has been associated with deficits in stress response and social behavior, as well as with strong effects on myelination, synaptic plasticity, neurotransmitter levels, and neurogenesis.[2] Microbiota changes have been reported in ASD patients, and ASD-related behavioral endophenotypes in mouse models are comorbid with microbiota alterations, immune dysfunction and deficient gastrointestinal integrity.[2] In a preclinical mouse model of ASD driven by maternal immune activation, microbiome manipulation in the offspring via treatment with human commensal *B. fragilis* resulted in improvements in anxiety-like and stereotyped behavior but not in sociability.[3] Moreover, BTBR mice — a widely used model of ASD, have reduced abundance of bile-metabolizing *Bifidobacterium* and *Blautia* bacterial species in the intestine and these changes in the gut microbiota were associated with marked gastrointestinal distress and reduced sociability.[4] In another model of ASD using maternal chronic high-fat diet as an insult, the offspring displayed deficits in social behavior and cognition that could be reversed by supplementation of another specific bacterial strain *L. reuteri*.[5] This restoration of behavioral deficits was associated with an increase in the expression of the prosocial hormone oxytocin in the hypothalamus and restitution of social interaction-induced synaptic potentiation in the dopaminergic reward linked ventral tegmental area (VTA). The *L. reuteri*-mediated rescue of social deficits was later established to be generalizable across different genetic (*Shank3B*$^{-/-}$), environmental (valproic acid during pregnancy) and idiopathic (BTBR) ASD models.[6] *L. reuteri*, in a vagus nerve-dependent manner, was found to rescue social interaction-induced synaptic plasticity in the VTA of ASD mice, but not in oxytocin receptor-deficient mice, demonstrating a direct link between *L. reuteri*-led behavioral rescue and host oxytocinergic signaling.

These last two studies from Costa-Mattioli lab describing *L. reuteri*-mediated reversal of social deficits in ASD models set the stage for their recently published paper,[7] where the authors focus on another genetic model of neurodevelopmental disorders (*Cntnap2*$^{-/-}$). Mouse knockout of the *Cntnap2* (contactin-associated protein-like 2) has been shown to exhibit core ASD-related phenotypes — defects in the migration of cortical projection neurons, reduced number of GABAergic interneurons, as well as social behavior and hyperactivity deficits.[8] Interestingly, previous studies have shown that administration of the antipsychotic risperidone ameliorates the repetitive behavior, but not the social deficits in *Cntnap2*$^{-/-}$ mice, a dissociation observed in human patients which hints mechanistic differences between the two hallmark behavioral signatures.[8] Building on this, the authors discover that the hyperactivity phenotype of *Cntnap2*$^{-/-}$ mice is caused by host genetics, whereas using germ-free (GF) animals (microbiota deficient) they show that the social behavior phenotype is mediated selectively by the gut microbiome.[7] Moreover, these latter effects can be reversed by *L. reuteri* administration.

In the study, the authors recapitulate these differential origins of the two core behaviors and demonstrate that mutant mice differ drastically from wild types in the gut microbiota composition including *L. reuteri* levels (Fig. 1). To illustrate that gut microbiota is the selective driver of the social deficits, the authors use multiple elegant approaches (including co-housing with isolated wild-type and non-transgenic littermates, separation experiments, microbiota transplantation into GF mice, selective *L. reuteri* reconstitution, and oxytocin administration), all converging on normal microbiota-dependent reversal of social deficits, while still maintaining the hyperactivity phenotype. To dig deeper into mechanisms, the authors performed faecal metabolomic profiling and found that *L. reuteri* boosts the host's endogenous BH4 (tetrahydrobiopterin, a co-factor involved in the biosynthesis of dopamine, serotonin, and nitric oxide) pathway. Remarkably, direct administration of BH4 to the

[1]APC Microbiome Ireland, University College Cork, Cork, Ireland and [2]Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland
Correspondence: John F. Cryan (j.cryan@ucc.ie)

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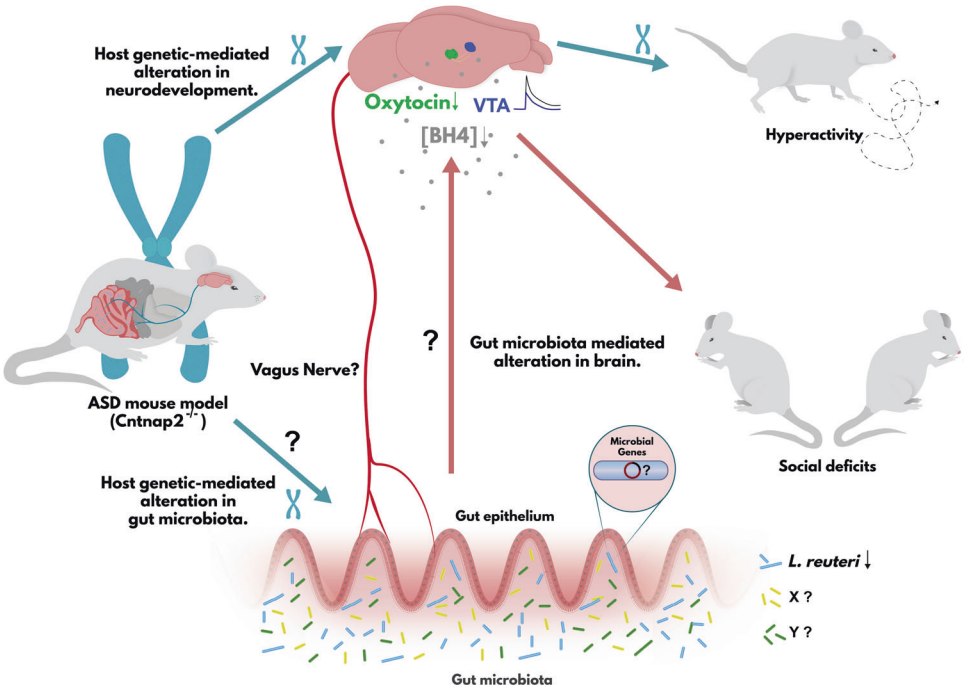

Fig. 1 Host genetics and microbiota differentially regulate behaviors in the *Cntnap2*$^{-/-}$ mouse model of ASD. The hyperactivity phenotype is caused by host genetics, whereas the social deficits are mediated by the gut microbiome. Selective administration of *L. reuteri* or the metabolite BH4 restores social behaviors, oxytocin levels and VTA plasticity. (The authors would like to thank P Venkata Atreya Sai for help with the illustration.)

animal's gut also rescues social behavior and related changes in synaptic function in VTA, without having any effects on hyperactivity phenotype. Interestingly, BH4 is already a known target in autism research with moderate success in clinical trials,[9] but several questions remain regarding BH4 and its impact on behavior (Fig. 1): What is the precise mechanism by which BH4 is produced and signals to the brain? Which other bacterial species either produce or induce BH4 and whether they can promote social behavior? And are the effects mediated by the vagus nerve? Further research is also warranted to determine how the loss of *Cntnap2* results in alterations in gut microbiota composition. Another avenue to consider the host genetics-microbiome crosstalk is their interactions in the critical windows of development of perinatal period and adolescence, time periods of specific vulnerability to neurodevelopmental disorders.[10] Could hyperactivity be rescued with microbiota-targeted interventions in an early critical period?

Overall, the big question that could reshape the way we look at brain health and disease is: Are these remarkable findings generalizable and can be extended to other complex behaviors and/or diseases? For instance, do other genetic variants regulate behavior and brain function via host genetics and microbiome interactions? Further extending the concept to an organismal level: are other diseases, such as obesity, cancer, metabolic disorders, also subject to differential host genetic-microbiome control? Given the millions of years of co-evolution of microbes and host cells, a hologenomic view of physiological processes where microbes and host are equal partners, is going to keep the research in health and disease in good stead.[11]

ADDITIONAL INFORMATION

Competing interests: J.F.C has been an invited speaker at meetings organized by Mead Johnson, Yakult, Alkermes, and Abbott Nutrition and has received research funding from Cremo, IFF, Pharmavite and Nutricia. He has been a consultant for Alkermes and Nestle. J.N. has no competing interests to declare.

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