Toward Effective Probiotics for Autism and Other Neurodevelopmental Disorders

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Hsaio and colleagues link gut microbes to autism spectrum disorders (ASD) in a mouse model. They show that ASD symptoms are triggered by compositional and structural shifts of microbes and associated metabolites, but symptoms are relieved by a *Bacteroides fragilis* probiotic. Thus probiotics may provide therapeutic strategies for neurodevelopmental disorders.

Rapid advances in analytical and sequencing technologies have spurred a renaissance of research into connections between the microbial communities that inhabit our gut and physiological conditions. Given the complexity of gut microbial communities, estimated to contain 500–1,000 species that considerably expand our metabolic potential beyond what the human genome encodes, it is perhaps unsurprising that they can influence many aspects of our physiology and gut-linked health and disease. For example, TLR5 knockout mice can become obese because an altered microbial community, instead of affecting metabolic efficiency, increases its appetite (Vijay-Kumar et al., 2010), or in a mouse model of multiple sclerosis, demyelination only occurs in the context of the gut microbiota (Lee et al., 2011, Berer et al., 2011). In addition, microbial impacts on neurology are also evident, including anxiety and sociability in mice (Collins et al., 2013) and changing emotions in humans who received fermented milk with probiotics (Tillisch et al., 2013). In this issue of *Cell*, Hsaio et al. (2013) make a striking contribution to our understanding of the influence of gut bacteria using an animal model that replicates autism-like behaviors in mouse offspring following maternal immune activation (Figure 1). They show that microbial shifts within the gut of a mouse resulted in changes of metabolites in the serum and that these lead to the onset of autism-like behaviors. Moreover, administering a beneficial bacterium, *Bacteroides fragilis*, reversed the physiological, neurological, and immunological anomalies.

Autism diagnoses have increased rapidly over the last decade (currently 1 in 88 births, versus 1 in 150 reported in 2000; http://www.cdc.gov/ncbddd/autism/data.html), but no clear relationships between genetic factors and ASD symptoms have yet been found. However, gastrointestinal ASD symptoms suggest a potential breakdown in normal symbiotic relationships between the host and its microbes (a dysbiosis), which may affect health via systemic as well as direct pathways, including immune system interactions. In some ASD cases, gut barrier integrity is reduced, increasing permeability. This condition, together with maternal immune activation (MIA), can increase the abundance of certain bacterial metabolites in the serum of offspring, which if aberrant could influence host behavior. Although current literature regarding microbiota associated with ASD patients is limited and contradictory, there is evidence that ASD patients lack certain beneficial bacteria in their gut, e.g., *Prevotella* (Kang et al., 2013).

To bring these issues into sharper focus in an experimentally tractable system, Hsaio and colleagues used the MIA paradigm to model autism-like behaviors in mice. In this animal model, pregnant mice were injected with an immunostimulant, polyinosinic:polycytidylic acid (poly(I:C)), which mimics a viral infection. MIA results in offspring with ASD-like behavioral symptoms and neuropathology. They showed that this mouse model for MIA reduced intestinal integrity through altered gut bacterial community. In offspring with reduced gut barrier integrity, the authors identified ~8% of assayed bacterial metabolites that differed significantly in abundance compared to those with intact gut barrier function. When the MIA offspring mice were fed with *Bacteroides fragilis*, a gut microbe with positive effects on the immune system, the abundance of 34% of these metabolites changed back, gut barrier integrity was improved, the gut-microbiome was restored to a state similar to control mice, and a number of ASD-related behavioral abnormalities were ameliorated. In addition, a 46-fold increase of 4-ethylphenylsulfate (4EPS) in the serum of MIA offspring returned to normal levels.

The authors demonstrated the gut bacteria may generate 4EPS by showing that germ-free mice have undetectable serum concentrations of 4EPS. Interestingly, 4EPS accumulates in patients with chronic renal failure and is related to *p*-cresol, which is present in urine of children with ASD and is suggested as a

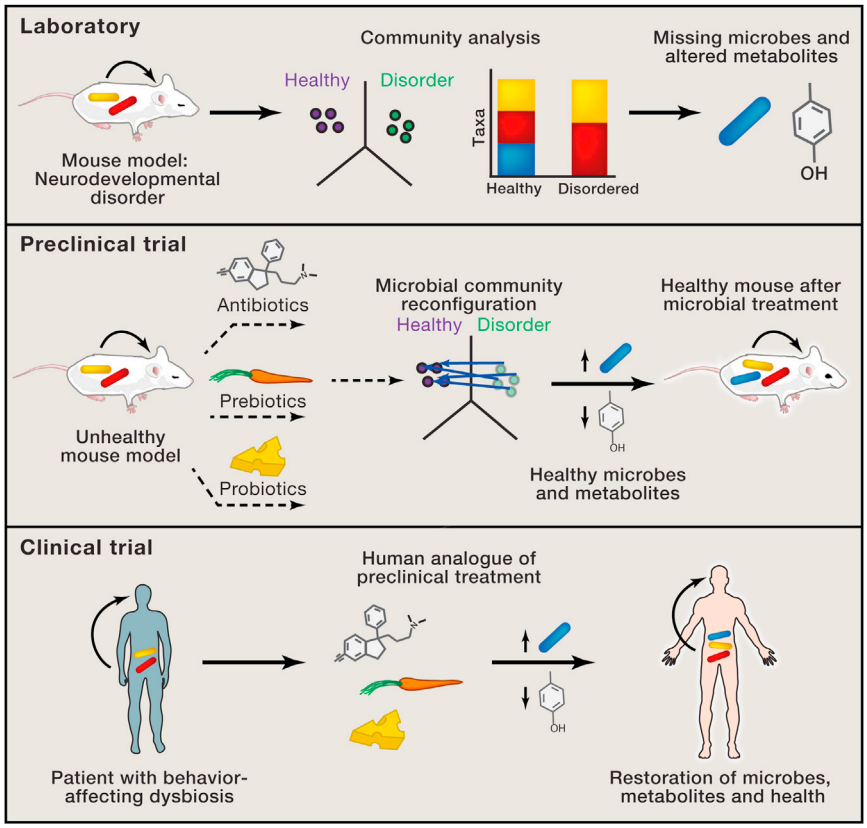

Figure 1. Evolution of a Pipeline for Therapeutic Strategies for Neurodevelopmental Disorders Based on Microbiome and Metabolite Profiling

Top: Experiments using mouse models (for example, an MIA mouse with symptoms of ASD and potentially other behavioral disorders) and subsequent community profiling can provide a mechanistic understanding of the importance of specific gut microbes and their metabolites in triggering the illness process, especially when lead compounds or microbes are applied to germ-free mice. Middle: Potential treatments to restore the healthy state of the mouse model (replacements of the identified missing microbes and/or the differences in metabolites they cause) can be tested and validated in preclinical trials, including different strategies for altering the microbial community and/or metabolite profile. For example, introducing a beneficial microbe such as the probiotic strains of *B. fragilis* used by Hsaio et al. may decrease a harmful metabolite rather than increasing a beneficial one. Bottom: Formulation and application of analogous treatments in human trials may lead to new ways to treat the behavioral and physiological problems associated with human neurodevelopmental disorders. Careful clinical trials will be needed in humans, as the effects of a given microbe and metabolite may differ in different species.

human autism biomarker (Persico and Napolioni, 2013), although additional studies would be needed to confirm the generality of this finding. Coincidently, the MIA mice in this study had *p*-cresol in their serum, but it was not at significant levels. When the authors added synthetic 4EPS to wild-type mice, they induced anxiety-like behavior similar to that observed in MIA mice. A second metabolite elevated in the MIA serum, and normalized by treatment with *B. fragilis*, was indolepyruvate. Indolepyruvate is generated by microbial tryptophan catabolism and is related to indolyl-3-acryloylglycine, another human autism marker. Indolepyruvate elevation could be linked to increased serum levels of serotonin, yet another human autism biomarker. Application of the *B. fragilis* probiotic increased many other metabolites, including N-acetylserine, which the authors hypothesize may provide protection against some ASD symptoms.

This groundbreaking study provides some of the first conclusive evidence of the impact of MIA on GI tract integrity that is reversible via administration of a specific probiotic. It also shows that a suite of metabolic markers is generated by bacteria, altered in dysbiosis, and normalized by probiotic treatment. Importantly, the authors demonstrate that elements of the MIA phenotype can be caused by a specific microbial metabolite. This is an excellent example of how a combination of bacterial community profiling, mouse models, germ-free mice, and metabolomics can be used to mechanistically understand the effects of the gut microbiome on health and disease states and to develop therapeutic strategies to treat key conditions.

The broader potential of this research is obviously an analogous probiotic that could treat subsets of individuals with ASD. The observation that 4EPS imparts anxiety-like symptoms in normal mice suggests that other neurodevelopmental illnesses may also be linked to microbial metabolites in serum. If probiotics, such as *B. fragilis* that ameliorate 'bad' metabolites along with their negative neurological consequences could be identified in relevant mouse models, the implications for the mental health of humans are extraordinary. MIA has been linked to a range of human conditions, including depression and schizophrenia (Knight et al., 2007), and several reports indicate that probiotics can treat anxiety and posttraumatic stress disorder (PTSD) in mouse models, including one model that requires an intact vagus nerve for gut-brain signaling (Bravo et al., 2011). Therapies that target our microbial side may hold the key to making progress against a wide range of notoriously difficult psychiatric illnesses.

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Probing DNA by 2-OG-Dependent Dioxygenase

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TET-mediated 5-methyl cytosine (5mC) oxidation acts in epigenetic regulation, stem cell development, and cancer. Hu et al. now determine the crystal structure of the TET2 catalytic domain bound to DNA, shedding light on 5mC-DNA substrate recognition and the catalytic mechanism of 5mC oxidation.

Substantial amounts of 5-hydroxymethyl cytosine (5hmC) have been observed in murine embryonic stem cells and brain. The three ten-eleven translocation (TET1-3) proteins have been identified to act as 2-oxoglutarate (2-OG)- and Fe(II)-dependent dioxygenases to catalyze the oxidation of 5mC to 5hmC. TET dioxygenases can further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxycytosine (5caC) in genomic DNA. 5caC can then be recognized by thymine DNA glycosylase in DNA demethylation processes (Kohli and Zhang, 2013). In this issue, Hu et al. (2013) determine the crystal structure of the TET2-DNA complex to elucidate how TET dioxygenases may mediate 5mC oxidation and the impact of TET2 clinical mutations.

Most missense TET2 mutations are in the catalytic domain. Hu et al. show that clinical mutations found in human cancers decrease catalytic activity by affecting the 5mC recognition, DNA interaction, and 2-OG/Fe(II) interactions in vitro and in vivo. Frequent mutations of TET2 in leukemia suggest that TET2 helps to regulate hematopoiesis and that mutations impact cancer development. Although multiple mutations may be needed to cause the clinical phenotype (Delhommeau et al., 2009), the TET2 structural analysis helps to define the connection between these mutations and disease phenotypes.

TET1−3 share similar catalytic activities and conserved C-terminal catalytic domains, which contain a Cys-rich region and double-strand β helix (DSBH) domain. The structure of TET2 catalytic domain with dsDNA reveals 5mC-DNA substrate recognition with bound Fe(II) and N-oxalylglycine (NOG, a 2-OG analog). The interesting intramolecular interaction of Cys-rich and DSBH domains includes an unexpected domain swap between these domains. This feature helps to create a unique holder for DNA substrate recognition, in which both the Cys-rich and DSBH domains bind DNA.

Only two other 2-OG-dependent dioxygenase-DNA complex structures, human ABH2 and *E. coli* AlkB, have been previously known. Human ABH2 dioxygenase can repair by oxidative demethylation several different methylated DNA modifications (1mA, 3mC, 1mG, and 3mT) (Yi et al., 2012). Due to the instability of base pairing, the methylated base is flipped out. ABH2 uses Phe102 to probe the base lesion on DNA, and Phe102 intercalates into the duplex stack to ensure that the methyl group is positioned in the catalytic cavity (Figure 1A) (Yang et al., 2008). According to this tipping mechanism (Figure 1A), the Phe102 tip constantly probes for base-pair instability as DNA slides through the ABH2 protein. Similarly, TET2 dioxygenase uses Tyr1294 to probe for base-pair instability, and this residue intercalates into the duplex stack when a methylated cytosine is found. Mutation of Tyr1294 results in decreased activity. Hu et al. show that there is no binding difference between 5mCpG-DNA and 5CpG-DNA, implying that the methyl group does not impact DNA binding. TET2 seems unlikely to discriminate 5hmC, 5fC, and 5caC in the active site as long as the 5mC derivatives