News & Views

MICROBIOME

**Targeting microbial metabolites to treat autism**

A first-in-class therapeutic that targets neuroactive microbial metabolites in the gut shows promising target engagement, safety, and behavioral improvements in adolescents with autism spectrum disorder.

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Autism spectrum disorder (ASD) is a group of heterogenous neurodevelopment conditions, characterized by deficits in social communication and interaction in conjunction with restricted, repetitive patterns of behaviors and interests. Many affected individuals experience gastrointestinal (GI) dysfunction, as well as a range of comorbidities including sleep disorders, epilepsy, and anxiety. Currently, there are no approved drugs for treating the core symptoms of ASD. Although the etiology remains poorly understood, it is widely recognized that genetic and environmental factors and their interactions contribute to ASD phenotypes. One such environmental risk factor is the gut microbiome, a key regulator of brain development and behavior1.

In this issue of *Nature Medicine*, Campbell *et al.* provide the first preliminary clinical evidence that AB-2004, a first-in-class, molecular therapeutic that prevents the absorption of neuroactive microbial metabolites from the GI tract, can help improve ASD-associated behaviors (Fig. 1). In this and a companion article by Needham et al (published in this issue of *Nature*), the group also describe preclinical studies in mouse models which provide the rationale for taking this therapeutic approach into the clinic.

There is growing evidence that specific gut microbiota-derived metabolites (termed neuroactive microbial metabolites) can cross the blood brain barrier2, and directly modulate neural networks involved in the control of affective, social, and cognitive processes3. A landmark preclinical study in 2013 was the first to link behavioral abnormalities relevant to ASD and other neurodevelopmental disorders with reduced gut barrier integrity and alterations in the gut microbiota – in particular, this study implicated the gut microbial metabolite 4-ethylphenyl sulfate (4EPS) in these behavioral phenotypes4. It was recently reported that 4EPS is also elevated in the serum of the CNTNAP2 genetic mouse model of ASD. In humans, there is growing evidence of dysregulation of 4EPS and several structurally related phenolic molecules in feces and plasma of individuals with ASD5,6. Interestingly, circulating 4EPS abundance appears to be particularly elevated in a subset of children with ASD and GI symptoms5, and mouse studies indicate that it likely contributes to atypical neurodevelopment in mammals.

In their study, Campbell *et al*. demonstrate the potential for orally administered porous carbon particles to improve ASD-associated behaviors by modifying host exposure to 4EPS and other gut-derived neuroactive metabolites. AB-2004 is a spherical carbon adsorbent that has high affinity for uremic toxins and related aromatic metabolites including those derived from, or modulated by, the gut microbiota – such as 4EPS, 4*-*cresyl-sulfate, 3-indoxyl sulfate and hippurate. AB-2004 can sequester these molecules in the gut, preventing their absorption and circulation, and is excreted in the feces (Fig. 1). By directly targeting gut microbiota-derived metabolites, this novel approach eliminates the need for a drug that crosses the blood-brain barrier and minimizes systemic side effects. Furthermore, its effectiveness is not influenced by the large amounts of inter-individual variation in gut microbial composition or functionality.

In a series of elegant experiments, Campbell *et al*., bioengineered mouse models that were selectively colonized with bacteria strains capable of robustly producing 4EP from dietary tyrosine (4EP+ mice) or with mutant strains (4EP- mice) lacking this ability, to mimic in a simplified model the ASD condition. The 4EP+ mice were found to excrete 4EPS in their urine and exhibited anxiety-like behavior. Cognitive and motor functions, however, were not modulated by 4EPS, indicating a selective effect of this phenolic metabolite on emotional behaviors. Crucially, reduced amounts of circulating 4EPS were observed in 4EP+ mice that received AB-2004 treatment in their regular diet and these animals did not exhibit anxiety-like behavior. In their parallel study, the same authors showed that 4EPS enters the brain, and modulates neural activity and functional connectivity within brain networks underlying emotion regulation. Consistent with previous studies linking gut microbiota to brain myelination7, 4EPS was observed to influence oligodendrocyte maturation and function. For example, 4EP+ mice showed disorganized myelin in the paraventricular nucleus of the thalamus, an important node in the emotional processing neuronal network. Remarkably, pharmacological treatment with clemastine fumarate, a drug that promotes oligodendrocyte differentiation, prevented 4EPS-induced anxiety-like behavior. These observations may have important clinical implications, since recent studies have identified a transcriptional signature implicating oligodendrocyte biology and myelination in ASD and altered patterns of functional brain connectivity have been strongly associated with behavioral features of ASD.

Encouraged by the above preclinical findings, Campbell *et al*. recruited 30 adolescents with a confirmed diagnosis of ASD and the presence of GI symptoms to participate in a phase 1b/2a open clinical trial of AB-2004 treatment. AB-2004 treatment was found to be safe, well-tolerated and without any major adverse effects, thus meeting the primary endpoints of their open-label clinical trial. Moreover, the results showed target engagement of AB-2004, as indicated by reductions in the target gut microbial-derived metabolites in the plasma and urine following 2-months of treatment, and a general rebound to baseline after cessation of treatment. Additionally, AB-2004 decreased the number of participants experiencing GI-related issues. The authors found signs of treatment efficacy across multiple exploratory behavioral endpoints, with most striking effects found in two comorbid domains of ASD, specifically in irritability and anxiety. Importantly, these behavioral effects were more pronounced in individuals with elevated baseline levels of irritability or anxiety scores. However, no correlations were observed between any single metabolite and behavior scores, suggesting potential interactions between multiple metabolites. In a subset of ten study participants, the authors also found changes in brain functional connectivity patterns following AB-2004 treatment in regions associated with emotional processes such as anxiety (i.e., amygdala and anterior cingulate cortex)8, consistent with findings from their preclinical studies. Given the heterogeneity in gene-environment interactions in ASD, it will be important for future studies to better characterize the metagenomic, genomic, immunological, and dietary factors underpinning treatment effectiveness.

Targeting neuroactive microbial metabolites is one of several strategies that have been employed to manipulate the gut microbiota-gut-brain axis. Other approaches have aimed to directly target the microbiome including profound modulation of these intestinal residents using antibiotics and fecal microbial transplants (FMT), and more subtle manipulation via nutritional strategies (e.g., prebiotics, probiotics, postbiotics) aimed at fortifying specific microbial groups or the synthesis to specific microbial products. Recently, more refined approaches have been developed to inhibit specific bacterial enzymes to block targeted activities. However, regarding ASD, successful examples have been largely limited to animal models, and current evidence supporting beneficial effects and long-term safety of these approaches in pediatric ASD are still limited. In addition, factors such as diet have been recently noted to obscure study outcomes.

The data presented by Campbell *et al.* and Needham et al.represents an important milestone in the study of the microbiota-gut-brain axis, as it delineates an innovative gut-restricted therapeutic strategy to improve some ASD-associated behaviors, namely irritability and anxiety. Although these behaviors are not considered core symptoms of ASD, they are common comorbid conditions in pediatric ASD and have major implications for ASD developmental trajectories and health-related quality of life of these patients. The current antipsychotic medications used to treat irritability behaviors are associated with a range of side effects, making it difficult for children to tolerate, especially in the long-term. Therefore, if AB-2004 treatment proves effective, safe, and well-tolerated in randomized double-blinded placebo-controlled trials, it could offer an exciting novel therapeutic approach for the ASD community.

Fig. 1. **Treatment with AB-2004 improves gastrointestinal problems and non-core behavioral symptoms of ASD**. The gut-brain axis is a bidirectional communication network connecting the brain and the gastrointestinal tract. Several pathways of communication have been implicated, including the production of gut bacterial-derived metabolites that directly influence the brain (so called neuroactive microbial metabolites) and subsequently behavior. Individuals with ASD exhibit elevated amounts of various metabolites such as 4-ethylphenyl sulfate (4EPS) and *p*-cresyl sulfate (pCS) in serum and feces. AB-2004 directly targets neuroactive microbial metabolites in the gut, diminishing systemic exposure and limiting their impact on the brain.

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**Competing interests**

The authors declare no competing interests