Published online: 21 March 2022
https://doi.org/10.1038/s41591-022-01743-0

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Acknowledgements
This research was supported in part by the National Human Genome Research Institute of the National Institutes of Health under awards RM1HG007257-07 and K01HG011683. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Competing interests
The authors declare no competing interests.

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.

Rochellys Diaz Heijtz, Pierre Gressens and Jonathan R. Swann

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 behavior[1].

In this issue of *Nature Medicine*, Campbell et al[2]. 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 a concurrent issue of *Nature*)[3], the group also describe preclinical studies in mouse models that provide a rationale for taking this therapeutic approach into the clinic.

There is growing evidence that specific metabolites derived from gut microbiota (termed neuroactive microbial metabolites) can cross the blood–brain barrier[4] and directly modulate neural networks involved in the control of affective, social and cognitive processes[3]. 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—implicating the gut microbial metabolite 4-ethylphenyl sulfate (4EPS), in particular, in these behavioral phenotypes[5]. It was recently reported that 4EPS is also elevated in the serum of the *CNTNAP2* genetic mouse model of ASD[3]. In humans, there is growing evidence of dysregulation of 4EPS and several structurally related phenolic molecules in the feces and plasma of individuals with ASD[6,7]. Interestingly, circulating 4EPS appears to be particularly elevated in a subset of children with ASD and GI symptoms[6], and mouse studies indicate that it is likely to contribute to atypical neurodevelopment in mammals[3].

In their study, Campbell et al[2]. demonstrate that orally administered porous carbon particles have the potential 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, *p*-cresyl sulfate, 3-indoxyl sulfate and hippurate. AB-2004 can sequester these molecules in the gut, preventing their absorption and circulation, and is then 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, minimizing 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[2]. selectively colonized mice with bacterial strains bioengineered to produce 4EP (4EP$^+$ mice) and compared them to mice colonized 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. Their cognitive and motor functions were unaffected, however, indicating that this phenolic metabolite has a selective effect 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[3], 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 myelination[8], they found that 4EPS influenced 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

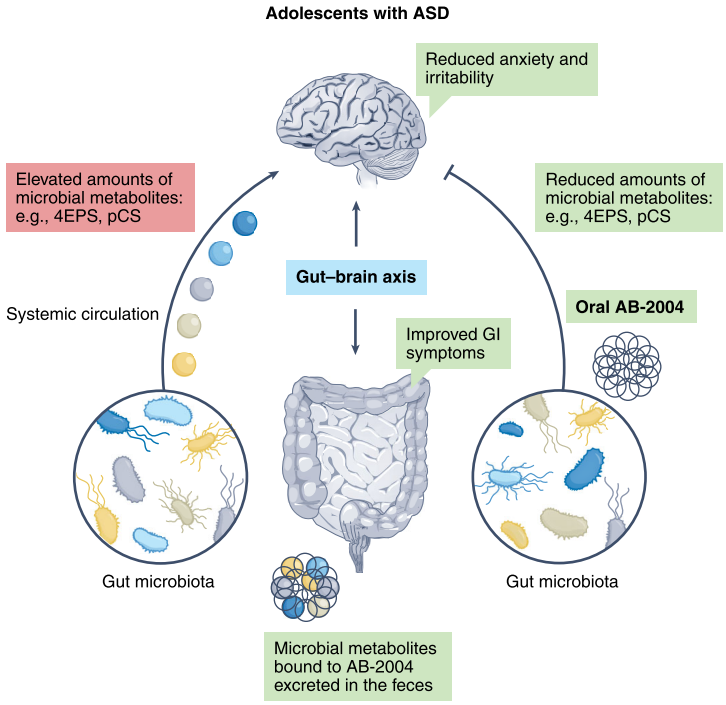

Fig. 1 | Treatment with AB-2004 improves gastrointestinal problems and non-core behavioral symptoms of ASD. The gut–brain axis is a bidirectional communication network connecting the central nervous system and the gastrointestinal tract. Several pathways of communication have been implicated, including the production of gut-bacterial-derived metabolites (so-called neuroactive microbial metabolites) that directly influence the brain 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.

clemastine fumarate, a drug that promotes oligodendrocyte differentiation, prevented 4EPS-induced anxiety-like behavior. These observations may have important clinical implications, as recent studies have identified a transcriptional signature implicating oligodendrocyte biology and myelination in ASD[9], and altered patterns of functional brain connectivity are strongly associated with behavioral features of ASD[10].

Encouraged by the above preclinical findings, Campbell et al[2]. recruited 30 adolescents with a confirmed diagnosis of ASD and GI symptoms to participate in a phase 1b/2a open-label clinical trial of AB-2004 treatment. They found AB-2004 to be well tolerated and without any concerning adverse effects, thus meeting the primary endpoints of the trial[2]. Moreover, the results showed target engagement of AB-2004, as indicated by reductions in the target microbial-derived metabolites in the plasma and urine after 2 months of treatment, and a general rebound to baseline after treatment cessation. Additionally, AB-2004 decreased the number of participants experiencing GI-related problems. The authors found signs of treatment efficacy across multiple exploratory behavioral endpoints, with the most striking effects relating to two comorbid domains of ASD, specifically irritability and anxiety. These behavioral effects were more pronounced in individuals with elevated baseline 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)[11], consistent with findings from their preclinical studies[3]. 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–brain axis. Other approaches have aimed to directly and profoundly target the gut microbiome using antibiotics and fecal microbial transplants, as well as more subtle manipulation via nutritional strategies (e.g., prebiotics, probiotics, postbiotics) aimed at fortifying specific microbial groups or promoting the synthesis of, or exposure to, specific microbial products. Recently, more refined approaches have been developed to inhibit specific bacterial enzymes to block targeted activities. However, in regard to ASD, successful examples have largely been limited to animal models, and current evidence supporting beneficial effects and long-term safety of these approaches in pediatric ASD are still limited[12]. In addition, factors such as diet have been recently noted to obscure study outcomes[13].

The data presented by Campbell et al[2]. and Needham et al[3]. represent an important milestone in the study of the microbiota–gut–brain axis, as they delineate 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 the ASD developmental trajectories and health-related quality of life of these patients. The antipsychotic medications currently used to treat irritability behaviors are associated with a range of side effects, making them difficult for children to tolerate, especially 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 new therapeutic approach for the ASD community. ❐

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Published online: 14 February 2022
https://doi.org/10.1038/s41591-022-01711-8

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Acknowledgements

R.D.H. is supported by the Swedish Research Council, the Swedish Brain Foundation, the Frimurare Barnhus Foundation and the European Union (ITN SmartAge, grant agreement number 859890). P.G. is supported by Inserm, the Université de Paris, the Horizon 2020 Framework Program of the European Union (grant agreement no. 874721/PREMSTEM), the ANR, the Fondation Grace de Monaco, the Fondation des Gueules Cassées and an additional grant from "Investissement d'Avenir-ANR-11-INBS-0011"–NeurATRIS. J.R.S. is supported by the National Institute for Health Research Southampton Biomedical Research Centre, Biotechnology and Biological Sciences Research Council (BB/W00139X/1) and Medical Research Council (MR/W003597/1).

Competing interests

The authors declare no competing interests.

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DIABETES

Tirzepatide for diabetes: on track to SURPASS current therapy

A prospective meta-analysis supports the cardiovascular safety of the dual incretin receptor agonist tirzepatide, after promising clinical outcomes in the SURPASS-4 study.

Filipe A. Moura, Benjamin M. Scirica and Christian T. Ruff

The elevated cardiovascular risk associated with type 2 diabetes is a major public health problem. Over the past decade, pharmacological management of diabetes has been transformed with the discovery of new classes of drugs that have demonstrated considerable cardiovascular benefit — particularly inhibitors of the sodium–glucose co-transporter SGLT2 and agonists of the incretin GLP-1 receptor. Each class seems to have a unique profile, with the former addressing predominantly heart failure and kidney disease[1,2] and the latter addressing atherothrombotic risk[3,4].

Tirzepatide is a dual agonist of GLP-1 and another incretin hormone, GIP. It is the first dual-incretin agent to be evaluated in phase 2 and 3 studies and demonstrates clinically impressive reductions in blood glucose levels[5] and weight[5]. Recently, results from the SURPASS-4 study suggested that tirzepatide was not associated with an increase in cardiovascular risk relative to the risk noted for insulin, in patients with established cardiovascular disease or at high risk for developing it[6]. In this issue of *Nature Medicine*, Sattar et al. extend the cardiovascular safety evaluation of tirzepatide in a broader population with type 2 diabetes by means of a pre-specified meta-analysis of individual patient data from seven clinical trials[7].

Incretins, first identified in the 1980s, are hormones secreted by the gut soon after eating that regulate insulin levels and satiety. Incretins GIP and GLP-1 both have important roles in normal glucose homeostasis (Fig. 1). In patients with diabetes, the pancreas becomes less responsive to incretins (predominately to GIP) and, over the past decade, pharmacological-level replacement of GLP-1 receptor agonism has become one of the most effective tools for improving glucose homeostasis, weight loss and cardiovascular risk in patients with diabetes. By comparison, GIP agonism appears to have a less insulinotropic effect, and early research suggested that standalone GIP was ineffective in patients with type 2 diabetes[8]. There were theoretical reasons, however, to believe that combining a GLP-1 receptor agonist with GIP would further increase insulin secretion in response to hyperglycemia in type 2 diabetes[9]. The effect of GIP on insulin secretion may be at least partially restored after several weeks of improvement in glucose levels[10]. Adipose tissue has extensive expression of GIP receptors, and GIP amplifies adipose-tissue sensitivity to insulin, facilitating the role of adipose tissue in lipid buffering after meals and thus preventing the deposition of fat outside of traditional fat deposits (with potential implications for overall insulin sensitivity and glucose control). GIP may also have a role in enhancing GLP-1-mediated central satiety[11]. Together, these data supported the development of tirzepatide as a treatment for patients with type 2 diabetes, culminating in the recent, promising SURPASS-4 study outcomes.

It was in this context that Sattar et al. carried out their meta-analysis to extend the cardiovascular safety evaluation of tirzepatide to a broader population with type 2 diabetes than those enrolled in each of the individual trials[7]. They pooled individual patient data from seven clinical trials that compared tirzepatide against placebo or additional diabetes pharmacotherapy in adults with type 2 diabetes that was inadequately controlled with lifestyle modification and/or a combination of well-established glucose-lowering drugs. Although these trials were primarily designed to evaluate the effect of tirzepatide on glucose control and weight loss, data on cardiovascular events were prospectively collected and in turn analyzed by Sattar et al[7]. The duration of six of these studies was less than 1 year, which is shorter than pivotal cardiovascular outcome clinical trials in type 2 diabetes (which generally accrue approximately 2 years of follow-up to achieve the requisite number of events). Only SURPASS-4 had a longer follow-up, with a median of 1.6 years. There were also significant differences between trials in baseline cardiovascular risk, with SURPASS-4 having the highest proportion of patients at high cardiovascular risk (86.9%, versus 10.6% in all other trials). Although the differences in trial design, duration and comparator arms present challenges in the analysis and interpretation of these pooled data, the current meta-analysis permits review of the totality of cardiovascular outcomes in the tirzepatide-development program. Overall, the pooled results from the group that