Cheung, K.J., Padmanaban, V., Silvestri, V., Schipper, K., Cohen, J.D., Fairchild, A.N., Gorin, M.A., Verdone, J.E., Pienta, K.J., Bader, J.S., and Ewald, A.J. (2016). Polyclonal breast cancer metastases arise from collective dissemination of keratin 14-expressing tumor cell clusters. Proc. Natl. Acad. Sci. USA *113*, E854–E863.

Conklin, M.W., Eickhoff, J.C., Riching, K.M., Pehlke, C.A., Eliceiri, K.W., Provenzano, P.P., Friedl, A., and Keely, P.J. (2011). Aligned collagen is a prognostic signature for survival in human breast carcinoma. Am. J. Pathol. *178*, 1221–1232.

Das, S., Shapiro, B., Vucic, E.A., Vogt, S., and Bar-Sagi, D. (2020). Tumor Cell-Derived IL1β Promotes Desmoplasia and Immune Suppression in Pancreatic Cancer. Cancer Res. *80*, 1088–1101.

Duda, D.G., Duyverman, A.M., Kohno, M., Snuderl, M., Steller, E.J., Fukumura, D., and Jain, R.K. (2010). Malignant cells facilitate lung metastasis by bringing their own soil. Proc. Natl. Acad. Sci. USA *107*, 21677–21682.

Dufies, O., and Boyer, L. (2021). RhoGTPases and inflammasomes: Guardians of effector-triggered immunity. PLoS Pathog. *17*, e1009504.

Fu, A., Yao, B., Dong, T., Chen, Y., Yao, J., Liu, Y., Li, H., Bai, H., Liu, X., Zhang, Y., et al. (2022). Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer. Cell *185*, 1356–1372.

Helmink, B.A., Khan, M.A.W., Hermann, A., Gopalakrishnan, V., and Wargo, J.A. (2019). The microbiome, cancer, and cancer therapy. Nat. Med. *25*, 377–388.

Massagué, J., and Obenauf, A.C. (2016). Metastatic colonization by circulating tumour cells. Nature *529*, 298–306.

Provenzano, P.P., Eliceiri, K.W., Campbell, J.M., Inman, D.R., White, J.G., and Keely, P.J. (2006). Collagen reorganization at the tumor-stromal interface facilitates local invasion. BMC Med. *4*, 38.

Trust your gut, lest thou be anxious

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Can gut-residing bacteria influence mood and anxiety? And can targeting bacteria-produced metabolites reduce anxiety? Based on two *Nature* and *Nature Medicine* papers, the answers to these questions are likely yes. Needham, Campbell, and colleagues identified bacteria that enhance anxiety-like behaviors in mice and ways to mitigate anxiety in autistic patients.

Gastrointestinal (GI) dysfunction is one of the comorbid symptoms of autism spectrum disorder (ASD) patients. Numerous studies suggest a potential link between the altered gut microbiome and ASD-related phenotypes. A recent study indicates that ASD patients' dietary preferences might contribute to differences in their microbial community compared to control subjects (Yap et al., 2021). On the other hand, a small-scale clinical study reported that ASD patients exhibited long-term benefits from fecal microbiota transplantation. This procedure relieved the patients' ASD-related symptoms, suggesting a causal relationship between the microbiota and ASD symptoms (Kang et al., 2019).

If gut bacteria affect brain function, as suggested by these studies, it is likely through three different routes: influencing gut-innervating neurons, educating immune cells, or producing metabolites (Huh and Veiga-Fernandes, 2020). Using a preclinical mouse model, the Mazmanian group previously found that systemic administration of a single bacterial metabolite, 4-ethylphenyl sulfate (4EPS), induced anxiety-like behaviors in mice (Hsiao et al., 2013). Furthermore, the plasma levels of 4EPS and other structurally related metabolites, such as cresol derivatives and 4-allylphenyl sulfate, were elevated in ASD patients (Needham et al., 2021). However, it was unknown which bacteria and bacterial enzymes produce 4EPS and whether these molecules contribute to ASD patients' anxiety behaviors.

To address these questions, Needham et al. first screened candidate gut bacterial isolates and identified two bacterial species, *Bacteroides ovatus* and *Lactobacillus plantarum*, that convert dietary tyrosine to the intermediates 4-vinylphenol and 4-etyhylphenol (4EP), respectively (Figure 1A) (Needham et al., 2022). Subsequently, they posited, 4EP is likely sulfated to 4EPS by the host enzyme sulfotransferase SULT1A1. Consistent with the idea that 4EPS synthesis requires bacterial enzyme activity, 4EP was not found in germ-free mice. Colonization of germ-free mice with an engineered *B. ovatus* (containing an extra copy of enzymes that produce 4-vinylphenol) and *L. plantarum* (facilitating the synthesis of 4EP) led to elevated levels of both 4EP and 4EPS in their feces, urine, and brain. Mice carrying these two bacteria, compared to control mice carrying bacteria that do not produce 4EP, displayed differences in functional connectivity in brain regions, including the hippocampus, thalamus, amygdala, and cortex. Furthermore, their phenotypes include altered oligodendrocyte maturation and an increased ratio of unmyelinated to myelinated axons in the brain, accompanied by the manifestation of anxiety-like behaviors.

Next, Campbell et al. designed a small-scale clinical trial in humans to investigate the modulatory function of 4EPS and related molecules in ASD

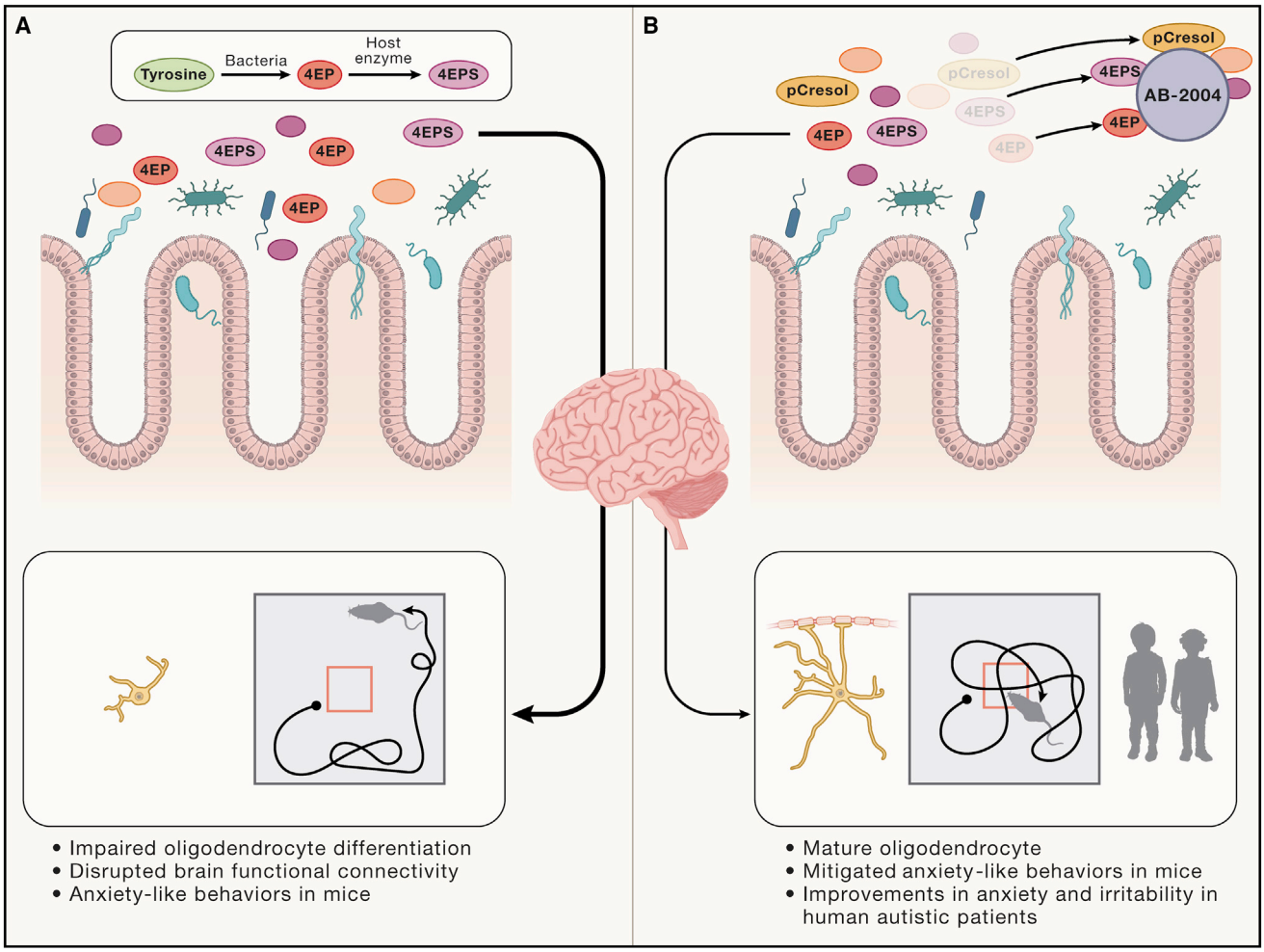

Figure 1. Bacteria-derived metabolites promote anxiety behaviors

(A) *Bacteroides ovatus* (*B. ovatus*) and *Lactobacillus plantarum* (*L. plantarum*) produce 4-ethylphenyl (4EP) from dietary tyrosine. 4EP is likely sulfated to 4-ethylphenyl sulfate (4EPS) by the host enzyme sulfotransferase, SULT1A1. The accumulation of 4EPS in the brain likely induces impaired oligodendrocyte differentiation, disrupts brain functional connectivity, and leads to anxiety-like behaviors.

(B) Oral administration of AB-2004 reduces levels of phenolic metabolites, including 4EPS, in the gastrointestinal tract and circulation. Administration of AB-2004 in mice results in proper oligodendrocyte maturation and reduced anxiety-like behaviors. Furthermore, AB-2004 mitigates anxiety and irritability phenotypes in autistic patients.

patients (Stewart Campbell et al., 2022). For this study, they used AB-2004, also known as AST-120, a drug developed in Japan 30 years ago to treat patients with chronic kidney problems and irritable bowel syndrome (Asai et al., 2019). Taken orally, AB-2004 binds and sequesters aromatic and phenolic compounds as it passes through the GI tract without being absorbed and is excreted along with bound molecules. Campbell et al. hypothesized that AB-2004 could reduce the systemic levels of 4EPS, *p*-cresol sulfate (pCS), and other related metabolites by sequestering them, reducing their amounts in the GI tract (Figure 1B). The open-label, phase 1b/2a clinical trial with 26 ASD patients indicated that AB-2004 treatments for 8 weeks decreased the urine and plasma levels of microbial metabolites, including 4EPS and pCS. Their levels rebounded to pre-treatment levels 4 weeks after completing the AB-2004 treatment. Consistent with preclinical data reported by Needham et al., AB-2004 administration altered brain connectivity between the amygdala and rostral anterior cingulate cortex in ASD patients, assessed by fMRI. Remarkably, AB-2004 reduced anxiety and irritability in ASD patients treated for 8 weeks. But the drug did not show sustained effects on anxiety 4 weeks after the cessation of the treatments.

Overall, these seminal papers provide insight into the mechanisms by which gut bacteria promote comorbid symptoms of ASD, such as anxiety and irritability, and ways to ameliorate these symptoms in human ASD patients. Of note, recent papers (Buffington et al., 2021; Wu et al., 2021), including one from the Mazmanian group, suggested that germ-free mice already display a core autism-like symptom—deficits in sociability—and the administration of "sociability-enhancing bacteria," including *Enterococcus faecalis* and *Lactobacillus*

reuteri, reversed these behavioral deficits. Thus, gut-bacteria can worsen one of the autism-related symptoms (e.g., anxiety) while mitigating one of the autism symptoms (e.g., impaired sociability). It is unclear what drives such seemingly contradictory outcomes or whether different bacteria contribute to distinct phenotypes. Since ASD is a highly heterogeneous disorder, it will be essential to interpret the bacterial modulation of autism-related phenotypes while also considering other genetic and environmental influences.

Future endeavors involving large-scale, double-blind, and placebo-controlled clinical studies will provide a better understanding of AB-2004's efficacy. We need to expand our arsenal beyond currently available FDA-approved drugs, such as aripiprazole and risperidone, to treatment strategies that improve autism-related comorbid symptoms. Given the rapid increase in autism prevalence, society desperately needs transformative ways to alleviate the core symptoms of ASD, including impairments in social interaction, deficits in communication, and repetitive behaviors. Perhaps this is just the beginning of what we can learn from gut-residing bacteria.

DECLARATION OF INTERESTS

J.R.H. is a co-founder of Interon laboratories. He is also a consultant for CJ Biosience.

REFERENCES

Asai, M., Kumakura, S., and Kikuchi, M. (2019). Review of the efficacy of AST-120 (KREMEZIN) on renal function in chronic kidney disease patients. Ren. Fail *41*, 47–56. https://doi.org/10.1080/0886022x.2018.1561376.

Buffington, S.A., Dooling, S.W., Sgritta, M., Noecker, C., Murillo, O.D., Felice, D.F., Turnbaugh, P.J., and Costa-Mattioli, M. (2021). Dissecting the contribution of host genetics and the microbiome in complex behaviors. Cell *184*, 1740–1756.e16. https://doi.org/10.1016/j.cell.2021.02.009.

Hsiao, E.Y., McBride, S.W., Hsien, S., Sharon, G., Hyde, E.R., McCue, T., Codelli, J.A., Chow, J., Reisman, S.E., Petrosino, J.F., et al. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell *155*, 1451–1463. https://doi.org/10.1016/j.cell.2013.11.024.

Huh, J.R., and Veiga-Fernandes, H. (2020). Neuroimmune circuits in inter-organ communication. Nat. Rev. Immunol. *20*, 217–228. https://doi.org/10.1038/s41577-019-0247-z.

Kang, D.W., Adams, J.B., Coleman, D.M., Pollard, E.L., Maldonado, J., McDonough-Means, S., Caporaso, J.G., and Krajmalnik-Brown, R. (2019). Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. Scientific Rep. *9*, 5821. https://doi.org/10.1038/s41598-019-42183-0.

Needham, B.D., Adame, M.D., Serena, G., Rose, D.R., Preston, G.M., Conrad, M.C., Campbell, A.S., Donabedian, D.H., Fasano, A., Ashwood, P., et al. (2021). Plasma and fecal metabolite Profiles in autism spectrum disorder. Biol. Psychiatry *89*, 451–462. https://doi.org/10.1016/j.biopsych.2020.09.025.

Needham, B.D., Funabashi, M., Adame, M.D., Wang, Z., Boktor, J.C., Haney, J., Wu, W.L., Rabut, C., Ladinsky, M.S., Hwang, S.J., et al. (2022). A gut-derived metabolite alters brain activity and anxiety behaviour in mice. Nature *602*, 647–653. https://doi.org/10.1038/s41586-022-04396-8.

Stewart Campbell, A., Needham, B.D., Meyer, C.R., Tan, J., Conrad, M., Preston, G.M., Bolognani, F., Rao, S.G., Heussler, H., Griffith, R., et al. (2022). Safety and target engagement of an oral small-molecule sequestrant in adolescents with autism spectrum disorder: an open-label phase 1b/2a trial. Nat. Med. *28*, 528–534. https://doi.org/10.1038/s41591-022-01683-9.

Wu, W.L., Adame, M.D., Liou, C.W., Barlow, J.T., Lai, T.T., Sharon, G., Schretter, C.E., Needham, B.D., Wang, M.I., Tang, W., et al. (2021). Microbiota regulate social behaviour via stress response neurons in the brain. Nature *595*, 409–414. https://doi.org/10.1038/s41586-021-03669-y.

Yap, C.X., Henders, A.K., Alvares, G.A., Wood, D.L.A., Krause, L., Tyson, G.W., Restuadi, R., Wallace, L., McLaren, T., Hansell, N.K., et al. (2021). Autism-related dietary preferences mediate autism-gut microbiome associations. Cell *184*, 5916–5931.e17. https://doi.org/10.1016/j.cell.2021.10.015.