

Transcriptomic Alteration in the Brain and Gut of Offspring Following Prenatal Exposure to Corticosterone

Eun-A Ko¹, Tong Zhou², Jae-Hong Ko^{3*} and Sung-Cherl Jung^{1*}

¹*Department of Physiology, College of Medicine, Jeju National University, Jeju 63243, Korea,*

²*Department of Physiology and Cell Biology, University of Nevada, Reno School of Medicine, Reno, NV 89557, USA,*

³*Department of Physiology, College of Medicine, Chung-Ang University, Seoul 06974, Korea*

Maternal stress during pregnancy can profoundly affect offspring health, increasing the risk of psychiatric disorders, metabolic diseases, and gastrointestinal problems. In this study, the effects of high prenatal corticosterone exposure on gene expression in the brain and small intestine of rat offspring were investigated via RNA-sequencing analysis. Pregnant rats were divided into two groups: Corti.Moms were injected with corticosterone daily, while Nor.Moms were given saline injections. Their offspring were labeled as Corti.Pups and Nor.Pups, respectively. The brain tissue analysis of Corti.Pups showed that the expression levels of the genes linked to neurodegenerative conditions increased and enhanced mitochondrial biogenesis, possibly due to higher ATP demands. The genes associated with calcium signaling pathways, neuroactive ligand-receptor interactions, and IgA production were also upregulated in the small intestine of Corti.pups. Conversely, the genes related to protein digestion, absorption, and serotonergic and dopaminergic synaptic activities were downregulated. These findings revealed that gene expression patterns in both the brain and intestinal smooth muscle of offspring prenatally exposed to corticosterone were substantially altered. Thus, this study provided valuable insights into the effects of prenatal stress on neurodevelopment and gut function.

Key words: Corticosterone, Neurodegenerative diseases, Brain-gut axis, Transcriptome

INTRODUCTION

Psychiatric disorders including attention deficit hyperactivity disorder (ADHD), autism spectrum disorder, bipolar disorder, depressive disorder, and schizophrenia, pose substantial challenges to health care systems, economies, and societies worldwide [1-4]. Studies have found that increased depressive symptoms in childhood behavioral issues in humans and animals are associated with maternal prenatal stresses, anxiety, or increased cortisol levels [5-11]. The hypothalamic-pituitary-adrenal (HPA) axis is essential for

regulating a range of responses, particularly stress. During pregnancy, the placenta functions as a crucial mediator between maternal and fetal systems, considerably influencing fetal development and maternal physiology. The HPA axis undergoes remarkable adaptations in which glucocorticoids are implicated in the complex interplay [12-15]. This proposed mechanism suggests that changes in these hormonal pathways, particularly in glucocorticoid levels or signaling, because of maternal stress or other factors may influence fetal development and contribute to the relationship between prenatal conditions and later psychiatric or neurodevelopmental outcomes [16-18].

The intricate relationship between psychiatric disorders and gastrointestinal (GI) function is mediated through the brain-gut axis, which facilitates complex bidirectional communication [19-23]. During periods of stress or anxiety, this axis can substantially influence the GI motor function. In response to emotional changes, this interaction often manifests as physical symptoms such as

Submitted November 7, 2024, Revised February 24, 2025,
Accepted February 26, 2025

*To whom correspondence should be addressed.
Sung-Cherl Jung, TEL: 82-64-754-3834, FAX: 82-64-702-2687
e-mail: jungsc@jejunu.ac.kr
Jae-Hong Ko, TEL: 82-2-820-5647, FAX: 82-2-817-7115
e-mail: akdongyi01@cau.ac.kr

diarrhea, dyspepsia, and abdominal discomfort [24–26]. The interplay between the central nervous system and the enteric nervous system (ENS) is also relevant in neurological conditions such as Parkinson's disease [27–30]. Therefore, a holistic approach that can manage conditions such as inflammatory bowel disease (IBD) and psychiatric disorders should be developed by considering mental health and GI function to optimize patient care and outcomes.

In our previous research, prenatal exposure to increased cortisol levels in pregnant rats (Corti.Moms; corticosterone-injected pregnant rat) has caused their offspring to exhibit ADHD-like behaviors (Corti.Pups; offspring exposed to elevated maternal corticosterone during pregnancy), including hyperactivity, impulsivity, and inattention [31]. Based on this finding, our current study aimed to explore the changes in the transcriptome underlying these behavioral and cognitive alterations induced by maternal prenatal stress. We conducted RNA sequencing (RNA-seq) on the brain tissue and the smooth muscle of the small intestine of Corti.Pups to investigate the transcriptomic changes associated with prenatal corticosterone exposure. Thus, we could gain further insights into the molecular mechanisms of prenatal stress-induced behavioral alteration and may recommend future strategies for the early intervention and treatment of stress-related neurodevelopmental disorders such as ADHD or disorder in GI functions.

MATERIALS AND METHODS

Animal model production

Female Sprague-Dawley rats were used from animal facility at Jeju National University Medical School. The experimental protocol, as previously described, involved two groups of pregnant rats [31]. The 8~9 week old pregnant rats received daily subcutaneous injections of corticosterone at a dose of 20 mg/kg throughout their pregnancy referred as Corti.Moms. The control group of pregnant rats received daily injections of saline (0.9% NaCl) instead of corticosterone, referred as Nor.Moms. The offspring of the Nor.Moms and Corti.Moms were designated as Nor.Pups and Corti.Pups, respectively. Corticosterone was suspended in 0.9% NaCl supplemented with 0.1% DMSO and 0.1% Tween 80, was used for injections. Brain and small intestine samples were collected from all male and female pups after they were sacrificed, following a two-week period of maternal care. To isolate smooth muscle from small intestine, the muscle layer was separated by sharp dissection on sylgard plate contained with ice-cold physiological solution. For bulk RNA-seq, the brain and smooth muscle of small intestine samples were immediately placed into TRIzol reagent.

RNA-seq data processing

The *kallisto* tool [32] was used to quantify the genome-wide gene expression profile from the fastq files based on the rat cDNA sequences obtained from the Ensembl database. The *edgeR* tool [33] was applied to perform the differential expression analysis between the Nor.Pup and Corti.Pup samples. The “TMM” method embedded in the *edgeR* tool was used for the normalization of the sequencing reads along with likelihood ratio test being used to identify the differentially expressed genes. The genes with the fold change (*FC*) greater than 1.5 and false discovery rate (*FDR*) less than 0.1 were deemed differentially expressed. Pathway/gene ontology analysis was performed based on the list of the differentially expressed genes using the DAVID online tool [34].

RESULTS

The differential expression of genes in the brain and small intestinal smooth muscle between the Nor.Pups and Corti.Pups

Volcano plots provide a visual representation of gene expression changes, illustrating the log₂-fold change between Nor.Pups and Corti.Pups (Fig. 1). This analysis revealed a substantial proportion of genes differentially expressed in both rat brain and small intestinal smooth muscle. In the brain, 60 genes showed differential expression, with 24 upregulated and 36 downregulated in the Corti.Pup group. The small intestine smooth muscle exhibited more extensive changes, with 108 differentially expressed genes, including 48 upregulated and 60 downregulated in Corti.Pups.

Among the upregulated genes in the brain of Corti.Pups, several mitochondrial genes associated with the electron transport chain, specifically Complex I components, were identified (Fig. 2). These included *Mt-nd1*, *Mt-4*, *Mt-nd4l*, *Mt-nd5*, *Mt-nd6*. The upregulation of these genes suggests enhanced mitochondrial activity and energy production in the brain tissue of Corti.Pups. Additionally, *Mt-atp8*, which encodes a subunit of ATP synthase (Complex V), was also upregulated, further supporting increased mitochondrial function and ATP production.

Fig. 3 is a heatmap generated from gene expression data related to differentially expressed genes in the small intestinal smooth muscle between Corti.Pups and Nor.Pups. In the small intestine of Corti.Pups, downregulation of several important transporter genes was observed (Fig. 3). Notably, *Slc1a1*, which encodes a glutamate was downregulated. Its downregulation in the intestine may indicate altered amino acid transport dynamics. Similarly, *Slc22a3*, which encodes the organic cation transporter 3, showed decreased expression. Another significant finding was the downregulation of *Scn1a* in the small intestine. This gene encodes the voltage-gated

11

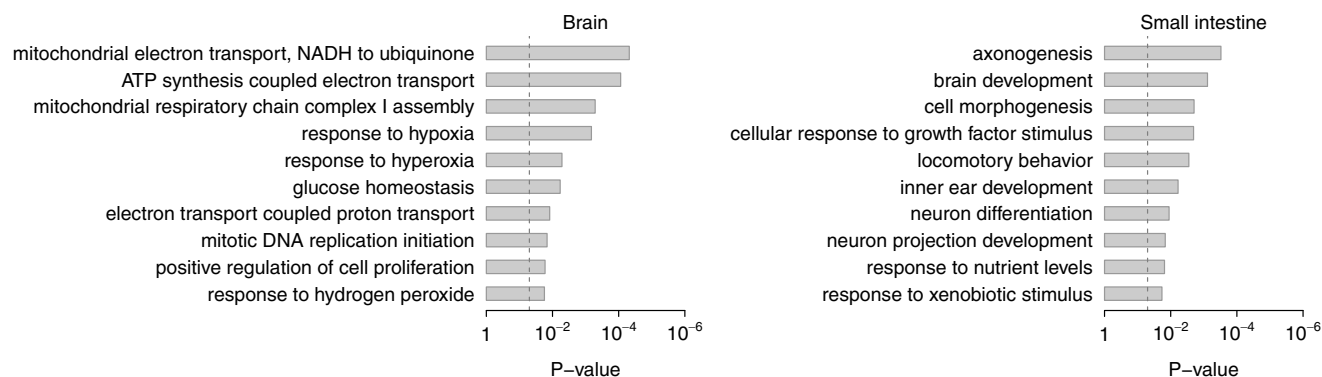


Fig. 4. The top ten GOBP terms associated with the differentially expressed genes in rat brains and intestinal smooth muscle. The vertical dash line indicates the p-value level of 0.05.

For example, spondins are known to be involved in cell adhesion and neurite outgrowth [35]. *Cxcr4* signaling is essential for proper formation of neural circuits by influencing neuronal migration and axon guidance during development [36]. *Hoxc6* is also known to influence neuronal differentiation and axon guidance in specific regions of the nervous system. *Hoxc6* could potentially play a role in establishing the ENS, which is a complex network of neurons within the intestinal smooth muscle [37]. While *Spon2*, *Cxcr4*, and *Hoxc6* have known roles in axonogenesis in general, whether or not they are involved in intestinal axonogenesis would require further research.

Comparison of signaling pathways between the Nor.Pups and Corti.Pups

Based on the lists of the differentially expressed genes, we further performed gene ontology/pathway analyses. The differentially expressed genes in the brains of the Corti.Pups (compared to Nor.Pups) revealed significant associations with several Gene ontology Biological Process (GOBP) terms. Fig. 4 represents the results of GOBP term enrichment analysis, highlighting biological processes significantly enriched in the brain and small intestine when comparing Corti.Pups to Nor.Pups. In the brain of Corti.Pups, enriched GOBP terms are associated with mitochondrial electron transport, ATP synthesis, response to hypoxia/hyperoxia, glucose homeostasis, cell proliferation, and DNA replication. Many of the top GOBP terms are directly linked to mitochondrial function and energy production, reflecting the brain's high energy demands. Additionally, terms such as response to hypoxia and response to hyperoxia indicate the brain's sensitivity to oxygen level fluctuations and its active response to oxidative stress. Overall, the dominant themes in the brain of Corti.Pups are energy metabolism and stress responses, particularly those related to oxygen and oxidative stress. In the small intestinal smooth muscle of Corti.

Pups, enriched GOBP terms include processes related to axonogenesis, brain development, cell morphogenesis, cellular response to growth factor stimulus, locomotory behavior, inner ear development, neuron differentiation, neuron projection development, response to nutrient levels, and response to xenobiotic stimulus. A notable feature is the presence of GOBP terms associated with neuronal development, reflecting the critical role of the ENS. In addition, we also tried a more stringent FDR cutoff of 0.05 to prioritize the differentially expressed genes. Gene ontology analysis reveals that the GOBP terms associated with new gene lists largely mirror the findings when a cutoff of 0.1 being used (Supplementary Fig. S1). These findings underscore the importance of neuronal growth, cell renewal, and responses to external stimuli such as nutrients, growth factors, and xenobiotics in the small intestine of Corti.Pups.

Fig. 5 represents a comparative analysis of KEGG pathway enrichment in the brain and small intestine of Corti.Pups relative to Nor.Pups. In the brain of Corti.Pups, pathways associated with neurodegenerative disease show the strongest enrichment, including Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease. This indicates significant dysregulation of genes involved in these disorders. The enrichment of oxidative phosphorylation pathways in the brain data is particularly noteworthy. This suggests potential alterations in mitochondrial function and energy metabolism, which are critical for normal neuronal activity and brain development [38]. Dysregulation of oxidative phosphorylation has been linked to the onset of cognitive decline and may be an early marker of neurodegeneration [39]. Additionally, the enrichment of pathways related to chemical carcinogenesis implies that genes involved in response to environmental toxins or cellular stress may also be affected in the brain of Corti.Pups. This could indicate altered neuroprotective mechanisms or increased susceptibility to cellular damage. On the other hand, the differ-

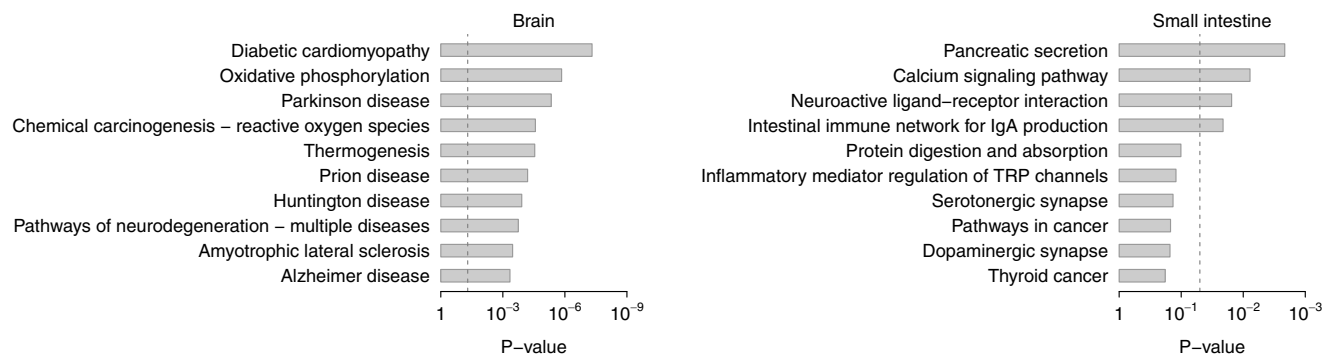


Fig. 5. The top ten KEGG pathways associated with the differentially expressed genes in rat brains and intestinal smooth muscle. The vertical dash line indicates the p-value level of 0.05.

entially expressed genes in rat small intestine showed significant enrichment in pathways such as calcium signaling pathway, neuroactive ligand–receptor interaction, intestinal immune network for IgA production, among others (Fig. 5 and Supplementary Table S1). Primarily enriched for pathways related to digestion, immune function, and cell signaling in Corti.Pups compared to Nor.Pups. In the small intestine, the enriched calcium signaling pathway likely modulates intestinal motility, secretion digestive enzymes, and nutrient absorption, as calcium signaling governs smooth muscle contraction, hormone secretion, and enzyme activation [40, 41]. The enriched neuroactive ligand–receptor interaction pathway suggests a significant role for neurotransmitters and neuropeptides in the intestinal environment. These interactions are particularly relevant because the ENS, the gut's intrinsic nervous system, uses these signals to regulate digestive processes [42, 43]. These findings highlight prenatal corticosterone treatment effects on energy metabolism and neurological disorders in the brain, while the small intestine exhibited alteration in calcium signaling and immune pathways.

DISCUSSION

During pregnancy, numerous factors, including maternal diet, maternal stress, depression, environmental toxins, and medication use, can influence neurodevelopmental and metabolic processes in the developing fetus [31, 44–48]. A previous study established remarkable links between these prenatal exposures and various outcomes, including an increased risk of ADHD and obesity/overweight among offspring [49, 50]. One of the major stress hormones released from maternal psychological stress is cortisol, which can be transferred to the fetus [14, 18, 51, 52]. Cortisol is a key regulator of stress-induced depression, and corticosterone injections in animal models have been shown to elevated plasma cortisol

levels, resulting in neuroendocrine dysregulation and depressive-like symptoms [53]. Typically, corticosterone is administered to pregnant rats to explore potential behavioral changes in their offspring that could be linked to neuropsychiatric disorders such as depression or autism. Surprisingly, in our previous study, rather than displaying the depressive-like behaviors commonly observed in adult models, the offspring of corticosterone-treated mothers exhibited hyperactivity, reduced anxiety, and impulsive behaviors [31]. To further explore this unexpected finding, the current study examines how maternal corticosterone exposure influences the transcriptome during early brain development and its potential impact on intestinal smooth muscle function via the brain–gut axis. Our transcriptomic analysis of the brain of Corti.Pups revealed several interesting findings such as a concerning pattern of the gene expression related to neurodegenerative diseases. Specifically, the genes associated with Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease were up-regulated. The increased expression of neurodegeneration-related genes suggested that maternal stress during pregnancy, as modeled prenatal corticosterone exposure, might potentially increase the offspring's susceptibility to these disorders later in life.

Neurological conditions are characterized by progressive neuronal loss and cognitive decline, which are often coupled with metabolic dysfunctions that affect glucose metabolism and mitochondrial biogenesis [54–60]. Notably, our transcriptomic analysis revealed that the mitochondrial-related genes in the brain of Corti.Pups were significantly upregulated compared with those of Nor.Pups. This finding suggested that prenatal stress might influence cellular energy metabolism. Specifically, we observed that the expression levels of several key mitochondrial genes in the brain tissue of Corti.Pups increased; these genes included those encoding NADH dehydrogenase subunits; *Mt-nd1*, *Mt-nd4*, *Mt-nd4l*, *Mt-nd5*, *Mt-nd6*, mitochondrial cytochrome b, and ATP synthase

subunits; and *Mt-atp6* and *Mt-atp8*. They are crucial components of the mitochondrial respiratory chain, which is essential for ATP production and overall energy metabolism [61-64]. Their upregulation suggests a potential compensatory response to increased energy demands or mitochondrial dysfunction induced by prenatal stress exposure. Neurons are dependent on the mitochondria for ATP production because of their high energy and oxygen consumption [65, 66]. Thus, they are more vulnerable to any kind of mitochondrial damage and dysfunction, which may trigger various neurodegenerative disorders [67, 68]. Their energy needs can be satisfied by enhancing mitochondrial function, potentially improving their survival and function [59, 60]. The increased mitochondrial gene expression observed in our study could indicate enhanced mitochondrial biogenesis, which was possibly triggered by the increased ATP demand. The compensatory upregulation of mitochondrial function is a vital adaptive response to increased energy demands, metabolic stress, and mitochondrial dysfunction. Cells can maintain energy homeostasis and support overall function by enhancing mitochondrial biogenesis, improving phosphorylation efficiency, and upregulating antioxidant defenses [69, 70].

Neurological conditions can remarkably disrupt the bidirectional communication between the gut and the brain, thereby changing intestinal sensitivity, motility, secretion, and permeability. As a result, a wide range of neurological and GI complications occur, emphasizing the intricate relationship between these systems [71-73]. For instance, patients with irritable bowel syndrome and IBD often suffer from psychological comorbidities, and the underlying mechanisms of these conditions involve complex interactions between the central nervous system, peripheral neurons, gut microbiota, and mucosal immune activation [74-76]. Our analysis of the small intestine of Corti.Pups compared with that of Nor.Pups revealed that the increased expression in calcium signaling and neuroactive ligand-receptor interactions could indicate that gut motility and sensitivity were altered. The upregulation of the IgA production network might imply that the intestinal immunity changed. Conversely, the decreased expression in protein digestion and absorption genes might indicate that nutrient processing capabilities were reduced. The downregulation of serotonergic and dopaminergic synapses could indicate that the gut-brain axis and ENS function were altered. Collectively, these changes highlighted the complex effect of prenatal stress on intestinal development and function. Serotonin, a major neurotransmitter in the ENS, can stimulate both excitatory and inhibitory enteric neurons, affecting motility patterns. It promotes peristalsis by stimulating intrinsic primary afferent neurons and can increase intestinal secretions and motility. Dopamine generally elicits an inhibitor effect on GI

motility. These neurotransmitters, along with the ENS, modulate gut function [77-79]. These findings suggest that prenatal exposure to corticosterone may substantially influence intestinal function, potentially altering signaling mechanisms, neuronal communication, and immune responses in the gut of the offspring.

Calcium dynamics are crucial for the proper functioning of intestinal smooth muscle. In the small intestinal smooth muscle of Corti.Pups, *Atp2b2*, which encodes plasma membrane calcium ATPase isoform 2 (PMCA2), plays a critical role in maintain these dynamics by transporting cytosolic calcium ions across the plasma membrane [80]. This process contributes helps regulate intracellular calcium levels, indirectly influencing motility and peristalsis [81]. In the colon, nitric oxide (NO), released by nitrergic neurons, serves as another key regulator of smooth muscle contraction and colonic migrating contractions through its interaction with interstitial cells of Cajal (ICCs) [82, 83]. Nitrergic neurons are essential for sustained smooth muscle relaxation, which is critical for propulsion and storage activities. Their inhibitory regulation ensure proper colonic motility and signal transmission. Interestingly, a reduced expression of *Nos1* (neuronal nitric oxide synthase) was observed in the small intestinal smooth muscle of Cori.Pups. *Nos1*, a primary source of NO in the ENS, acts as an important inhibitory neurotransmitter [84, 85]. The decreased expression of *Nos1* may impair local NO production, potentially contributing to motility disorders in the GI tract. Additionally, differences in the expression of *Myo16* and *Myo19* were noted. While distinct in function, both genes are critical for cellular processes. *Myo16* primarily operates in neuronal cells, where it regulates cytoskeleton dynamics and synaptic organization. It localizes to postsynaptic dendritic spines and modulates actin dynamics to influence synaptic structure and function. In contrast, *Myo19*, a mitochondrial motor protein, is involved in mitochondrial distribution and homeostasis. Together, these proteins are essential for maintaining cellular functionality. The synaptotagmin family members *Syt13* and *Syt15* also exhibited differential expression. *Syt13* is a multifunctional protein involved in pancreatic endocrine development, vesicle trafficking via calcium-independent mechanisms, and neuroendocrine signaling. It also has potential implications in cancer biology [86, 87]. Unlike most synaptotagmins that rely on calcium binding for their function, *Syt13* lacks calcium sensitivity due to structural differences in its C2A and C2B domains [88]. Its expression in enteroendocrine cells suggests a role in regulatory secretory pathways [87]. On the other hand, *Syt15* likely contributes to secretory vesicle trafficking and exocytosis, particularly in non-neuronal tissues. It may also act as a calcium sensor for membrane fusion processes; however, its precise role requires further investigation [89].

Our findings reveal significant transcriptomic changes in the

brains of offspring exposed to maternal corticosterone, particularly in upregulated neurodegenerative processes, energy metabolism, and stress response mechanisms. These results emphasize the importance of further investigating these pathways, as they may uncover potential therapeutic targets or early biomarkers for neurodegenerative diseases. Moreover, the observed impact on differentially expressed genes in intestinal smooth muscle could provide valuable insights into ENS development and GI function through the brain-gut axis. This highlights the interconnectedness of neural and digestive systems and opens avenues for exploring their mutual influences. It's important to note that our study, like many bioinformatics analyses, faces inherent challenges in translating computational predictions into biological systems. While large-scale gene expression analyses serve as powerful tools for generating hypotheses, experimental validation remains crucial.

ACKNOWLEDGEMENTS

SJ and JK conceived the idea. TZ performed the statistical analysis. EK and SJ prepared animal models and collected tissues. SJ, JK, EK, and TZ wrote the manuscript. All authors read and approved the final manuscript.

SJ was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (2022R111A3063177). JK was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2017R1D1A1B06035273). EK was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (NRF-2022R1F1A1062897).

REFERENCES

1. Cortese S, Coghill D, Fegert JM, Mattingly GW, Rohde LA, Wong ICK, Faraone SV (2025) Reply: the inclusion of methylphenidate in the WHO list of essential medicines is endorsed by millions of people with ADHD. *Eur Child Adolesc Psychiatry* 34:369-371.
2. Chen M, Tang Y, Li G, Xu MZ, Hou CL (2024) Case report: manic-like symptoms mimicking early-onset bipolar disorder in a case of neuropsychiatric lupus. *Bipolar Disord* 26:835-838.
3. Martinez M, Cai T, Yang B, Zhou Z, Shankman SA, Mittal VA, Haase CM, Qu Y (2024) Depressive symptoms during the transition to adolescence: left hippocampal volume as a marker of social context sensitivity. *Proc Natl Acad Sci U S A* 121:e2321965121.
4. Kojima Y, Yamada S, Kamijima K, Kogushi K, Ikeda S (2024) Burden in caregivers of patients with schizophrenia, depression, dementia, and stroke in Japan: comparative analysis of quality of life, work productivity, and qualitative caregiving burden. *BMC Psychiatry* 24:591.
5. Niknazar S, Nahavandi A, Najafi R, Danialy S, Zare Mehrjerdi F, Karimi M (2013) Parents' adulthood stress induces behavioral and hormonal alterations in male rat offspring. *Behav Brain Res* 252:136-143.
6. Zijlmans MA, Riksen-Walraven JM, de Weerth C (2015) Associations between maternal prenatal cortisol concentrations and child outcomes: a systematic review. *Neurosci Biobehav Rev* 53:1-24.
7. Rinne GR, Hartstein J, Guardino CM, Dunkel Schetter C (2023) Stress before conception and during pregnancy and maternal cortisol during pregnancy: a scoping review. *Psychoneuroendocrinology* 153:106115.
8. Baibazarova E, van de Beek C, Cohen-Kettenis PT, Buitelaar J, Shelton KH, van Goozen SH (2013) Influence of prenatal maternal stress, maternal plasma cortisol and cortisol in the amniotic fluid on birth outcomes and child temperament at 3 months. *Psychoneuroendocrinology* 38:907-915.
9. Slopen N, Roberts AL, LeWinn KZ, Bush NR, Rovnaghi CR, Tylavsky F, Anand KJS (2018) Maternal experiences of trauma and hair cortisol in early childhood in a prospective cohort. *Psychoneuroendocrinology* 98:168-176.
10. Axelrod J, Reisine TD (1984) Stress hormones: their interaction and regulation. *Science* 224:452-459.
11. Swales DA, Stout-Oswald SA, Glynn LM, Sandman C, Wing DA, Davis EP (2018) Exposure to traumatic events in childhood predicts cortisol production among high risk pregnant women. *Biol Psychol* 139:186-192.
12. Cottrell EC, Seckl JR (2009) Prenatal stress, glucocorticoids and the programming of adult disease. *Front Behav Neurosci* 3:19.
13. Hagaman AK, Baranov V, Chung E, LeMasters K, Andrabi N, Bates LM, Rahman A, Sikander S, Turner E, Maselko J (2020) Association of maternal depression and home adversities with infant hypothalamic-pituitary-adrenal (HPA) axis biomarkers in rural Pakistan. *J Affect Disord* 276:592-599.
14. Khoury JE, Giles L, Kaur H, Johnson D, Gonzalez A, Atkinson L (2023) Associations between psychological distress and hair cortisol during pregnancy and the early postpartum: a meta-analysis. *Psychoneuroendocrinology* 147:105969.
15. Serravalle L, Trespalacios F, Ellenbogen MA (2024) Hypothalamic-pituitary-adrenal axis functioning in offspring of parents with a major affective disorder: a meta-analytic review.

- Eur Child Adolesc Psychiatry.
16. Buss C, Entringer S, Moog NK, Toepfer P, Fair DA, Simhan HN, Heim CM, Wadhwa PD (2017) Intergenerational transmission of maternal childhood maltreatment exposure: implications for fetal brain development. *J Am Acad Child Adolesc Psychiatry* 56:373-382.
 17. Graham AM, Rasmussen JM, Entringer S, Ben Ward E, Rudolph MD, Gilmore JH, Styner M, Wadhwa PD, Fair DA, Buss C (2019) Maternal cortisol concentrations during pregnancy and sex-specific associations with neonatal amygdala connectivity and emerging internalizing behaviors. *Biol Psychiatry* 85:172-181.
 18. Davis EP, Head K, Buss C, Sandman CA (2017) Prenatal maternal cortisol concentrations predict neurodevelopment in middle childhood. *Psychoneuroendocrinology* 75:56-63.
 19. Moulton CD, Pavlidis P, Norton C, Norton S, Pariente C, Hayee B, Powell N (2019) Depressive symptoms in inflammatory bowel disease: an extraintestinal manifestation of inflammation? *Clin Exp Immunol* 197:308-318.
 20. Gracie DJ, Hamlin PJ, Ford AC (2019) The influence of the brain-gut axis in inflammatory bowel disease and possible implications for treatment. *Lancet Gastroenterol Hepatol* 4:632-642.
 21. Kurina LM, Goldacre MJ, Yeates D, Gill LE (2001) Depression and anxiety in people with inflammatory bowel disease. *J Epidemiol Community Health* 55:716-720.
 22. Roderburg C, Yaqubi K, Konrad M, May P, Luedde T, Kostev K, Loosen SH (2024) Association between inflammatory bowel disease and subsequent depression or anxiety disorders - a retrospective cohort study of 31,728 outpatients. *J Psychiatr Res* 169:231-237.
 23. Aziz Q, Thompson DG (1998) Brain-gut axis in health and disease. *Gastroenterology* 114:559-578.
 24. Zhu L, Huang D, Shi L, Liang L, Xu T, Chang M, Chen W, Wu D, Zhang F, Fang X (2015) Intestinal symptoms and psychological factors jointly affect quality of life of patients with irritable bowel syndrome with diarrhea. *Health Qual Life Outcomes* 13:49.
 25. Mao Y, Zhang P, Sun R, Zhang X, He Y, Li S, Yin T, Zeng F (2023) Altered resting-state brain activity in functional dyspepsia patients: a coordinate-based meta-analysis. *Front Neurosci* 17:1174287.
 26. Pollard KL, Campbell C, Squires M, Palsson O, van Tilburg MAL (2018) Seasonal association of pediatric functional abdominal pain disorders and anxiety. *J Pediatr Gastroenterol Nutr* 67:18-22.
 27. Wu JH, Guo Z, Kumar S, Lapuerta P (2011) Incidence of serious upper and lower gastrointestinal events in older adults with and without Alzheimer's disease. *J Am Geriatr Soc* 59:2053-2061.
 28. Khodabakhsh P, Bazrgar M, Dargahi L, Mohagheghi F, Asgari Taei A, Parvardeh S, Ahmadiani A (2021) Does Alzheimer's disease stem in the gastrointestinal system? *Life Sci* 287:120088.
 29. Shankle WR, Landing BH, Ang SM, Chui H, Villarreal-Engelhardt G, Zarow C (1993) Studies of the enteric nervous system in Alzheimer disease and other dementias of the elderly: enteric neurons in Alzheimer disease. *Mod Pathol* 6:10-14.
 30. Singh A, Dawson TM, Kulkarni S (2021) Neurodegenerative disorders and gut-brain interactions. *J Clin Invest* 131:e143775.
 31. Jeon SC, Kim HJ, Ko EA, Jung SC (2021) Prenatal exposure to high cortisol induces ADHD-like behaviors with delay in spatial cognitive functions during the post-weaning period in rats. *Exp Neurobiol* 30:87-100.
 32. Bray NL, Pimentel H, Melsted P, Pachter L (2016) Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol* 34:525-527.
 33. Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26:139-140.
 34. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, Imamichi T, Chang W (2022) DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res* 50:W216-W221.
 35. Klar A, Baldassare M, Jessell TM (1992) F-spondin: a gene expressed at high levels in the floor plate encodes a secreted protein that promotes neural cell adhesion and neurite extension. *Cell* 69:95-110.
 36. Lieberam I, Agalliu D, Nagasawa T, Ericson J, Jessell TM (2005) A Cxcl12-CXCR4 chemokine signaling pathway defines the initial trajectory of mammalian motor axons. *Neuron* 47:667-679.
 37. Beck F (2002) Homeobox genes in gut development. *Gut* 51:450-454.
 38. Rangaraju V, Lewis TL Jr, Hirabayashi Y, Bergami M, Motori E, Cartoni R, Kwon SK, Courchet J (2019) Pleiotropic mitochondria: the influence of mitochondria on neuronal development and disease. *J Neurosci* 39:8200-8208.
 39. Alldred MJ, Lee SH, Stutzmann GE, Ginsberg SD (2021) Oxidative phosphorylation is dysregulated within the basocortical circuit in a 6-month old mouse model of down syndrome and Alzheimer's disease. *Front Aging Neurosci* 13:707950.
 40. Perrino BA (2011) Regulation of gastrointestinal motility by

- Ca²⁺/calmodulin-stimulated protein kinase II. *Arch Biochem Biophys* 510:174-181.
41. Cho H, Lim J (2024) The emerging role of gut hormones. *Mol Cells* 47:100126.
 42. Sharkey KA, Mawe GM (2023) The enteric nervous system. *Physiol Rev* 103:1487-1564.
 43. Fung C, Vanden Berghe P (2020) Functional circuits and signal processing in the enteric nervous system. *Cell Mol Life Sci* 77:4505-4522.
 44. Soualeh N, Dridi I, Eppe G, Némós C, Soulimani R, Bouayed J (2017) Perinatal programming of depressive-like behavior by inflammation in adult offspring mice whose mothers were fed polluted eels: gender selective effects. *Brain Behav Immun* 63:137-147.
 45. Penteado SH, Teodorov E, Kirsten TB, Eluf BP, Reis-Silva TM, Acenjo MK, de Melo RC, Suffredini IB, Bernardi MM (2014) Prenatal lipopolysaccharide disrupts maternal behavior, reduces nest odor preference in pups, and induces anxiety: studies of F1 and F2 generations. *Eur J Pharmacol* 738:342-351.
 46. Wei S, Li Z, Ren M, Wang J, Gao J, Guo Y, Xu K, Li F, Zhu D, Zhang H, Lv R, Qiao M (2018) Social defeat stress before pregnancy induces depressive-like behaviours and cognitive deficits in adult male offspring: correlation with neurobiological changes. *BMC Neurosci* 19:61.
 47. Oh J, Woo HG, Kim HJ, Park J, Lee M, Rahmati M, Rhee SY, Min C, Koyanagi A, Smith L, Fond G, Boyer L, Kim MS, Shin JI, Lee SW, Yon DK (2024) Prenatal and infant exposure to antibiotics and subsequent risk of neuropsychiatric disorders in children: a nationwide birth cohort study in South Korea. *Psychiatry Res* 340:116117.
 48. Hmila I, Hill J, Shalaby KE, Ouararhni K, Abedsselem H, Modaresi SMS, Bihagi SW, Marques E, Sondhi A, Slitt AL, Zawia NH (2024) Perinatal exposure to PFOS and sustained high-fat diet promote neurodevelopmental disorders via genomic reprogramming of pathways associated with neuro-motor development. *Ecotoxicol Environ Saf* 272:116070.
 49. Cortese S, Tessari L (2017) Attention-deficit/hyperactivity disorder (ADHD) and obesity: update 2016. *Curr Psychiatry Rep* 19:4.
 50. Cortese S, Moreira-Maia CR, St Fleur D, Morcillo-Peñalver C, Rohde LA, Faraone SV (2016) Association between ADHD and obesity: a systematic review and meta-analysis. *Am J Psychiatry* 173:34-43.
 51. Field T, Diego M (2008) Cortisol: the culprit prenatal stress variable. *Int J Neurosci* 118:1181.
 52. Rehman KS, Sirianni R, Parker CR Jr, Rainey WE, Carr BR (2007) The regulation of adrenocorticotrophic hormone receptor by corticotropin-releasing hormone in human fetal adrenal definitive/transitional zone cells. *Reprod Sci* 14:578-587.
 53. Nazzari S, Fearon P, Rice F, Dottori N, Ciceri F, Molteni M, Frigerio A (2019) Beyond the HPA-axis: exploring maternal prenatal influences on birth outcomes and stress reactivity. *Psychoneuroendocrinology* 101:253-262.
 54. Zhong Y, Wang TH, Huang LJ, Hua YS (2024) Association between metabolic syndrome and the risk of Parkinson's disease: a meta-analysis. *BMC Neurol* 24:313.
 55. Minhas PS, Jones JR, Latif-Hernandez A, Sugiura Y, Durairaj AS, Wang Q, Mhatre SD, Uenaka T, Crapser J, Conley T, Ennerfelt H, Jung YJ, Liu L, Prasad P, Jenkins BC, Ay YA, Matrongolo M, Goodman R, Newmeyer T, Heard K, Kang A, Wilson EN, Yang T, Ullian EM, Serrano GE, Beach TG, Wernig M, Rabinowitz JD, Suematsu M, Longo FM, McReynolds MR, Gage FH, Andreasson KI (2024) Restoring hippocampal glucose metabolism rescues cognition across Alzheimer's disease pathologies. *Science* 385:eabm6131.
 56. Mori R, Yamamoto R, Shinzawa M, Otsuki N, Matsumura Y, Nakamura Y, Li Q, Sakaguchi Y, Matsui I, Mizui M, Shinomiya H, Ishibashi C, Nakanishi K, Kanayama D, Nagatomo I (2024) Breakfast frequency and smoking initiation in university students: a retrospective cohort study. *Nutrients* 16:2361.
 57. Ashleigh T, Swerdlow RH, Beal MF (2023) The role of mitochondrial dysfunction in Alzheimer's disease pathogenesis. *Alzheimers Dement* 19:333-342.
 58. Pantiya P, Thonusin C, Chattipakorn N, Chattipakorn SC (2020) Mitochondrial abnormalities in neurodegenerative models and possible interventions: focus on Alzheimer's disease, Parkinson's disease, Huntington's disease. *Mitochondrion* 55:14-47.
 59. Trigo D, Avelar C, Fernandes M, Sá J, da Cruz E Silva O (2022) Mitochondria, energy, and metabolism in neuronal health and disease. *FEBS Lett* 596:1095-1110.
 60. Dagar S, Sharma M, Tsapralis G, Tapia CS, Crynen G, Joshi PS, Shahani N, Subramaniam S (2024) Ribosome profiling and mass spectrometry reveal widespread mitochondrial translation defects in a striatal cell model of Huntington disease. *Mol Cell Proteomics* 23:100746.
 61. Aboulmaouhib B, Kastenmüller G, Suhre K, Zöllner S, Weissensteiner H, Prehn C, Adamski J, Gieger C, Wang-Sattler R, Lichtner P, Strauch K, Flaquer A (2022) First mitochondrial genome-wide association study with metabolomics. *Hum Mol Genet* 31:3367-3376.
 62. McBride HM, Neuspiel M, Wasiak S (2006) Mitochondria:

- more than just a powerhouse. *Curr Biol* 16:R551-R560.
63. La Morgia C, Caporali L, Gandini F, Olivieri A, Toni F, Nassetti S, Brunetto D, Stipa C, Scaduto C, Parmeggiani A, Tonon C, Lodi R, Torroni A, Carelli V (2014) Association of the mtDNA m.4171C>A/MT-ND1 mutation with both optic neuropathy and bilateral brainstem lesions. *BMC Neurol* 14:116.
 64. Tauchmannová K, Pecinová A, Houštěk J, Mráček T (2024) Variability of clinical phenotypes caused by isolated defects of mitochondrial ATP synthase. *Physiol Res* 73(Suppl 1):S243-S278.
 65. Yang W, Lei X, Liu F, Sui X, Yang Y, Xiao Z, Cui Z, Sun Y, Yang J, Yang X, Lin X, Bao Z, Li W, Ma Y, Wang Y, Luo Y (2024) Meldonium, as a potential neuroprotective agent, promotes neuronal survival by protecting mitochondria in cerebral ischemia-reperfusion injury. *J Transl Med* 22:771.
 66. Sayehmiri F, Motamedi F, Batool Z, Naderi N, Shaerzadeh F, Zoghi A, Rezaei O, Khodaghali F, Pourbadie HG (2024) Mitochondrial plasticity and synaptic plasticity crosstalk; in health and Alzheimer's disease. *CNS Neurosci Ther* 30:e14897.
 67. Panchal K, Tiwari AK (2019) Mitochondrial dynamics, a key executioner in neurodegenerative diseases. *Mitochondrion* 47:151-173.
 68. Gu W, Cong X, Pei Y, Che Ajuyo NM, Min Y, Wang D (2024) Impaired mitochondrial energy metabolism regulated by p70S6K: a putative pathological feature in Alzheimer's disease. *Metabolites* 14:369.
 69. Reddy PH (2009) Role of mitochondria in neurodegenerative diseases: mitochondria as a therapeutic target in Alzheimer's disease. *CNS Spectr* 14(8 Suppl 7):8-13.
 70. Federico A, Cardaioli E, Da Pozzo P, Formichi P, Gallus GN, Radi E (2012) Mitochondria, oxidative stress and neurodegeneration. *J Neurol Sci* 322:254-262.
 71. Ke S, Guimond AJ, Tworoger SS, Huang T, Chan AT, Liu YY, Kubzansky LD (2023) Gut feelings: associations of emotions and emotion regulation with the gut microbiome in women. *Psychol Med* 53:7151-7160.
 72. Michels N, Van de Wiele T, Fouhy F, O'Mahony S, Clarke G, Keane J (2019) Gut microbiome patterns depending on children's psychosocial stress: reports versus biomarkers. *Brain Behav Immun* 80:751-762.
 73. Camilleri M (2021) Gastrointestinal motility disorders in neurologic disease. *J Clin Invest* 131:e143771.
 74. Goodoory VC, Mikocka-Walus A, Yiannakou Y, Houghton LA, Black CJ, Ford AC (2021) Impact of psychological comorbidity on the prognosis of irritable bowel syndrome. *Am J Gastroenterol* 116:1485-1494.
 75. Zhao G, Lu Z, Liao Y, Sun Y, Zhang Y, Kang Z, Feng X, Sun J, Yue W (2024) Association of intestinal anti-inflammatory drug target genes with psychiatric disorders: a Mendelian randomization study. *J Adv Res*. doi: 10.1016/j.jare.2024.05.002.
 76. Fairbrass KM, Costantino SJ, Gracie DJ, Ford AC (2020) Prevalence of irritable bowel syndrome-type symptoms in patients with inflammatory bowel disease in remission: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 5:1053-1062.
 77. Mawe GM, Hoffman JM (2013) Serotonin signalling in the gut--functions, dysfunctions and therapeutic targets. *Nat Rev Gastroenterol Hepatol* 10:473-486.
 78. Shajib MS, Baranov A, Khan WI (2017) Diverse effects of gut-derived serotonin in intestinal inflammation. *ACS Chem Neurosci* 8:920-931.
 79. Ming X, Gao S, Sun J, Zhang N, Guo R, Feng X, Luan X, Xing H, Jiao Y, Guo F (2024) Regulation of the MCHergic neural circuit to dorsal raphe nucleus on emotion-related behaviors and intestinal dysfunction in mice model of irritable bowel syndrome with diarrhea. *Neuroendocrinology* 114:605-622.
 80. Vicario M, Zanni G, Vallese F, Santorelli F, Grinzato A, Cieri D, Berto P, Frizzarin M, Lopreiato R, Zonta F, Ferro S, Sandre M, Marin O, Ruzzene M, Bertini E, Zanotti G, Brini M, Cali T, Carafoli E (2018) A V1143F mutation in the neuronal-enriched isoform 2 of the PMCA pump is linked with ataxia. *Neurobiol Dis* 115:157-166.
 81. Howard A, Legon S, Walters JR (1993) Human and rat intestinal plasma membrane calcium pump isoforms. *Am J Physiol* 265:G917-G925.
 82. Wu Z, Wang Q, Yang F, Wang J, Zhao Y, Perrino BA, Chen J (2023) Functional and transcriptomic characterization of postnatal maturation of ENS and SIP syncytium in mice colon. *Biomolecules* 13:1688.
 83. Lies B, Gil V, Groneberg D, Seidler B, Saur D, Wischmeyer E, Jiménez M, Friebe A (2014) Interstitial cells of Cajal mediate nitroergic inhibitory neurotransmission in the murine gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol* 307:G98-G106.
 84. Takahashi T (2003) Pathophysiological significance of neuronal nitric oxide synthase in the gastrointestinal tract. *J Gastroenterol* 38:421-430.
 85. Rivera LR, Poole DP, Thacker M, Furness JB (2011) The involvement of nitric oxide synthase neurons in enteric neuropathies. *Neurogastroenterol Motil* 23:980-988.
 86. Tarquis-Medina M, Scheibner K, González-García I, Bastidas-Ponce A, Sterr M, Jaki J, Schirge S, García-Cáceres C, Lickert H, Bakhti M (2021) Synaptotagmin-13 is a neuroen-

- docrine marker in brain, intestine and pancreas. *Int J Mol Sci* 22:12526.
87. Bakhti M, Bastidas-Ponce A, Tritschler S, Czarnecki O, Tarkis-Medina M, Nedvedova E, Jaki J, Willmann SJ, Scheibner K, Cota P, Salinno C, Boldt K, Horn N, Ueffing M, Burtscher I, Theis FJ, Coskun Ü, Lickert H (2022) Synaptotagmin-13 orchestrates pancreatic endocrine cell egression and islet morphogenesis. *Nat Commun* 13:4540.
88. von Poser C, Südhof TC (2001) Synaptotagmin 13: structure and expression of a novel synaptotagmin. *Eur J Cell Biol* 80:41-47.
89. Siebenkäs C, Chiappinelli KB, Guzzetta AA, Sharma A, Jeschke J, Vatapalli R, Baylin SB, Ahuja N (2017) Inhibiting DNA methylation activates cancer testis antigens and expression of the antigen processing and presentation machinery in colon and ovarian cancer cells. *PLoS One* 12:e0179501.