Unveiling sex-based differences in developing propionic acid-induced features in mice as a rodent model of ASD

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ABSTRACT

Background. Males are more likely to develop autism as a neurodevelopmental disorder than females are, although the mechanisms underlying male vulnerability are not fully understood. Therefore, studying the role of autism etiologies considering sex differences in the propionic acid (PPA) rodent model of autism would build greater understanding of how females are protected from autism spectrum disorder, which may be used as a treatment strategy for males with autism.

Objectives. This study aimed to investigate the sex differences in oxidative stress, glutamate excitotoxicity, neuroinflammation, and gut microbiota impairment as etiological mechanisms for many neurological diseases, with specific reference to autism.

Method. Forty albino mice were divided into four groups of 10 animals each with two control and two treated groups of both sexes received only phosphate-buffered saline or a neurotoxic dose of PPA (250 mg/kg body weight) for 3 days, respectively. Biochemical markers of energy metabolism, oxidative stress, neuroinflammation, and excitotoxicity were measured in mouse brain homogenates, whereas the presence of pathogenic bacteria was assessed in mouse stool samples. Furthermore, the repetitive behavior, cognitive ability, and physical-neural coordination of the animals were examined.

Results. Collectively, selected variables related to oxidative stress, glutamate excitotoxicity, neuroinflammation, and gut bacteria were impaired concomitantly with altered behavior in PPA-induced rodent model, with males being more susceptible than females.

Conclusion. This study explains the role of sex in the higher vulnerability of males to develop autistic biochemical and behavioral features compared with females. Female sex hormones and the higher detoxification capacity and higher glycolytic flux in females serve as neuroprotective contributors in a rodent model of autism.

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INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that impairs brain development and typically manifests within the first three years of life (*Estes et al., 2015*). The reported incidence of ASD has risen dramatically over the last decade to one in 54 children and is more prevalent in boys at a 4:1 ratio (*Maenner et al., 2020*). ASD is distinguished by impaired social communication and repetitive and restrictive behavior (*Baio et al., 2018*). Treatments to cure the primary clinical symptoms of ASD remain unavailable. Although the exact etiological mechanisms of ASD are difficult to define, it is agreed that the occurrence of several or all the numerous factors comprising genetic, immune, nutritional, and environmental factors could contribute to the etiology of ASD (*Abuaish et al., 2021a*; *Abuaish et al., 2021b*; *Zurita et al., 2020*; *Sealey et al., 2016*; *Kreiser & White, 2014*).

Antibiotic overuse causes dysbiosis and inflammation that contributes to the pathophysiology of gastrointestinal diseases and autistic traits (*Navarro, Liu & Rhoads, 2016*). To create ASD-like animal models, propionic acid (PPA), a gut metabolic end product, can be administered and causes oxidative stress, mitochondrial dysfunction, neuroinflammation, and abnormal neurobehaviors as well as repetitive and poor social interactions in the rodent model (*Abuaish et al., 2021a*; *Choi et al., 2018*; *Nankova et al., 2014*; *El-Ansary, Bacha & Kotb, 2012*; *MacFabe et al., 2011*; *Shultz et al., 2008*).

The gut microbiota is a mutually beneficial bacterial habitat that is required for host survival and has a significant impact on the brain and behavior (*Fattorusso et al., 2019*; *Ristori et al., 2019*; *Coretti et al., 2018*) *via* the bidirectional gut-brain axis (GBA) that connects the gut to the central nervous system (CNS) (*Martin et al., 2018*; *Borrelli et al., 2016*). The GBA is reported to play an important role in social behavior management and in regulating myelin in the prefrontal cortex, a brain area that controls cognitive behavior (*Bastiaanssen & Cryan, 2021*; *Roman et al., 2018*). Males and females have sexually dimorphic patterns in energy and nutritional requirements throughout their lives, and therefore, gender differences in the gut microbiome-brain axis may be an important biological factor (*Bolnick et al., 2014*; *Schnorr et al., 2014*; *Kaplan et al., 2000*).

Oxidative stress occurs is caused by a lack of balance between the generation of reactive oxygen/nitrogen species (ROS) and the blocking of an damaging impacts through the use of antioxidative systems (*Pangrazzi, Balasco & Bozzi, 2020*). Oxidative stress is strongly considered to play a significant role in ASD pathophysiology (*Bjørklund et al., 2020*; *Nadeem et al., 2019*; *Wang et al., 2018*), and oxidative stress is critical to neuroinflammatory response, which has always been considered as a pathogenic factor of ASD (*Herbert, 2011*). *Rose et al. (2017)* found that children with autism are more vulnerable to oxidative stress because of an imbalance in glutathione (GSH) levels inside or outside of cells and a reduced GSH storage capacity compared with that in healthy siblings (*Rose et al., 2017*; *Hu et al., 2020*; *Bjørklund et al., 2016*). Estradiol is a steroidal hormone that plays a critical role in mitochondrial function and neuronal signaling and has been lately discovered to act as a neuroprotectant only under specific conditions (*Engler-Chiurazzi, Singh & Simpkins, 2016*; *Bruce-Keller et al., 2000*; *Lebesgue et al., 2009*). A key protective action of estradiol is the

ability to scavenge free radicals (*Prokai-Tatrai et al., 2009*; *Simpkins et al., 2010*), and this antioxidant activity has been linked to a sex difference in oxidative stress. Oxidative damage to DNA and lipid was found to be higher in the liver mitochondria and synapse of male rats than that in females (*Borrás et al., 2003*).

Glutamate (Glu) is the main excitatory neurotransmitters in the CNS, where it is involved in signal transmission and has a role in memory, learning, and synaptic plasticity (*Parkin et al., 2018*). However, high levels of extracellular Glu are linked to neuronal death, and Glu concentration is controlled by astrocytic Glu transporters, which remove Glu from the synapse after impulse transmission (*Jack et al., 2018*; *Jia et al., 2015*). By contrast, the main inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), causes hyperpolarization of the postsynaptic cell membrane, dampening the action potential (*El-Ansary et al., 2017*; *Ford, Nibbs & Crewther, 2017*). Increased levels of Glu are found with decreased levels of GABA and glutamine (Gln) and the Glu/GABA and Glu/Gln ratios in the plasma and brains of children with autism (*El-Ansary et al., 2017*; *Tu, Chen & He, 2012*). Investigations on sex differences in excitotoxicity have been performed by studying the neuro-protective effects of estrogen and prolactin as female sex hormones against hippocampal neurodegeneration (*Morales et al., 2014*; *Tejadilla, Cerbón & Morales, 2010*). Prolactin treatment in ovariectomized females has been indicated to minimize hippocampus neurodegeneration (*Morales et al., 2014*; *Tejadilla, Cerbón & Morales, 2010*). Particularly during phases of dynamic hormonal change, such as puberty, testosterone may have a key impact in how ASD manifests and multiple studies have suggested that estrogens are potent antioxidants that can prevent oxidative damage in cell culture systems and may inhibit Glu-induced excitotoxicity (*Silva et al., 1999*; *Muscatello et al., 2022*).

Neuroinflammation is a chronic glial reaction occurring in the CNS that can cause brain damage by enhancing proinflammatory cytokine release and abnormal neuronal growth (*Goines & Ashwood, 2013*; *Qasem, Al-Ayadhi & El-Ansary, 2016*). Analysis of interleukins (IL) has demonstrated that enhanced inflammatory activity is present in children with autism. Levels of proinflammatory cytokines were found to be higher in the plasma of children with ASD in comparison with those in age-matched healthy children and children with other developmental disabilities. This phenomenon is linked to behavioral impairments, implying that dysfunctional immune responses may affect core behaviors in ASD (*Ashwood et al., 2011*).

Various factors may cause brain-based sex differences in ASD, for instance, endocrine and genetic mechanisms that "masculinize" and "feminize" the brain at early development, puberty, and other life-cycle hormone transition periods. Moreover, the brain's arousal system and the stress axis may interact with sex-related biology to cause different neurodevelopmental patterns in females and males with ASD.

This information prompted us to investigate the gender differences in a number of biochemical and behavioral factors, including gut microbiota, oxidative stress, Glu excitotoxicity, and neuroinflammation in an ASD mouse model, to help understand why males are more likely than females to develop autistic traits and the protective mechanisms present in females. The current mouse model, differs from our previously used rat models (*Abuaish et al., 2021a*; *Abuaish et al., 2021b*; *El-Ansary, Bacha & Kotb,*

2012) and has been crucial in advancing our understanding of the behavioral impairments and physiology linked to sex differences in ASD and has enabled us to prepare the foundations of future research and new therapeutic approaches. We hypothesized that mice may make favorable models because they exhibit behavioral patterns and differences in most brain areas that are comparable with those reported in people with autism (*Kazdoba et al., 2016*).

As a preliminary investigation in mice with an ASD mouse model, sex differences were examined in relation to behavioral abnormalities in both sexes parallel with levels of oxidative stress, Glu signaling, neuroinflammation, and alterations to gut bacteria as ascertained with etiological mechanisms in ASD (*Nadeem et al., 2019*; *Wang et al., 2018*; *Ford, Nibbs & Crewther, 2017*; *Aabed et al., 2019*).

MATERIAL AND METHODS

Animal experiments

A total of 40 young (2–3-week-old) male and female albino mice (*Mus musculus*) weighing 13–15 and 11–13 g, respectively, were kept in cage ($40\times 5\times 20$ cm^3) at a controlled temperature (21 ± 1 °C) under standard laboratory conditions (humidity 37% and light for 12 h) with ad libitum access to standard laboratory animal feed pallets and water. Mice were obtained from the animal house in the Zoology Department in Science College, King Saud University, Riyadh, KSA. The experimental procedure was preapproved by the ethics committee for animal research of King Saud University, Riyadh (ethics reference number: KSU-SE-19-92).

Mice were divided into four groups of 10 animals each with two control and two treated groups of both sexes receiving phosphate-buffered saline (PBS) or an oral neurotoxic dose (250 mg/kg body weight) of PPA for 3 days, respectively (Fig. 1).

At the end of the experiment, mice were anesthetized using carbon dioxide and decapitated. Then, the whole brains of all groups were dissected into small pieces after being removed from the skull. Brain tissue (0.1 g) was rinsed with PBS and homogenized in one mL of PBS and kept at −20 °C.

Biochemical analyses

Catalase and glutathione-S-transferase (GST) activities were assessed according to the methods of *Maehly & Chance (1954)* and *Habig, Pabst & Jakoby (1974)*, respectively. Lactate dehydrogenase (LDH) and creatine kinase (CK) total activities were assessed using diagnostic kits made by the United Diagnostics Industry, Dammam, KSA (*Szasz, Gruber & Bernt, 1976*; *Amador, Dorfman & Wacker, 1963*). The level of GSH in the brain samples was measured according to the protocol described by *Beutler, Duron & Kelly (1963)* using a UV-visible spectrophotometer (Ultraspec 2100 Pro, Amersham Biosciences, Amersham, United Kingdom).

IL-6 (Catalog No MBS824703, MyBioSource, San Diego, CA, USA), IL-10 (Catalog No MBS704754, MyBioSource), Glu (Catalog No MBS2601720, MyBio-Source), and GABA (Catalog No MBS723819, MyBioSource) were investigated using enzyme-linked immunosorbent assay kits following the manufacturers' instructions. All measurements
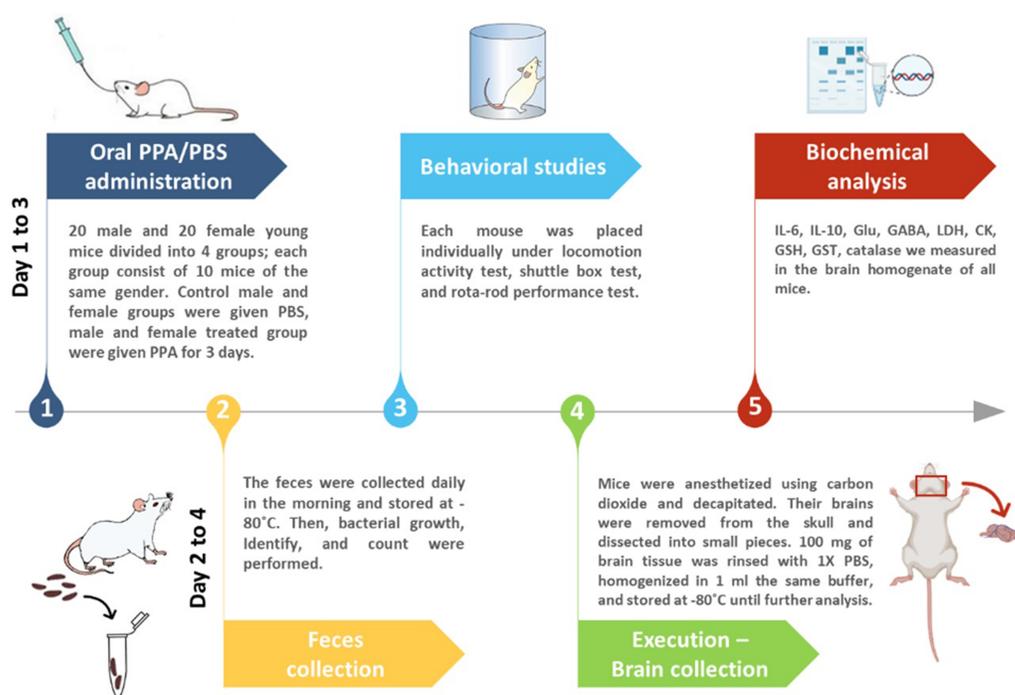
Figure 1 **Diagrammatic scheme of the animal experiments.**

were performed at least in duplicate, and the mean of different readings was calculated. Quality control assays were performed to evaluate the experimental reproducibility through the inter- and intra-assay coefficients of variability (%CV).

Microbial analysis

Sample collection: The fecal contents of all groups were collected on a daily basis in the morning in sterile tubes and immediately stored at −80 °C. To isolate and determine the bacterial etiologies of an infection as quickly and precisely as possible, bacteria were cultured utilizing the best artificial media and incubation conditions.

Techniques of bacterial culture

Fecal specimens were homogenized for 5 s in a vortex mixer (Intalb, Santa Clara, CA, USA) before being centrifuged for 3 min at 4,000 rpm at −4 °C (AllegraTM 21R, Beckman, Brea, CA, USA). Following centrifugation, tenfold serial dilutions of the fecal suspensions were performed by adding one mL of the supernatant to nine mL of PBS (dilution 1). The process was repeated until dilution 4 was reached. Then, 0.1 mL of each of the prepared dilutions was smeared on the surface of five different culture media: nutrient agar (NA) for the isolation of *Staphylococcus* and/or *Bacilli* (gram-positive or -negative rod), Macconkey agar (MCA) for distinguishing *Enterobacteriacea* (gram-negative rod, lactose fermenters), Mueller Hinton agar (MHA) to identify *Moraxella* spp. (gram-negative), blood agar for distinguishing gram-positive/negative rod and cocci, and sulfite polymyxin sulfadiazine (SPS) agar to distinguish *Clostridium botulinum*. NA, MCA, MHA, and blood agar plates

were incubated at 37 °C for 24 h under aerobic conditions, whereas SPS agar plates were incubated at 35 ± 2 °C for 24–48 h anaerobically (*Li et al., 2015*).

Quantification

Data from the culture-based methods were quantified based on a 1–4 scale defined as colony-forming unit (CFU), a measure of viable bacterial or fungal numbers. Unlike direct microscopic counts where all cells, dead and living, were counted, the CFU only measures viable cells. The number of CFUs on the plates was counted per dilution. Numbers between 30 and 300 were used to estimate the cultural count.

Identification

Every well-isolated colony, regardless of appearance, was picked in succession. If more than one colony type or more than one morphotype was observed, an attempt was made to isolate and characterize each of these. Each isolate was characterized according to the *Holdeman, Moore & Cato (1977)* procedure. Colonies were spread on the slide. Smears were heat-fixed and gently gram stained using 70% alcohol, crystal violet, safranin, and iodine. Slides were examined under a microscope using an oil immersion lens to identify these strains as gram-positive (violet color) or gram-negative (red color) and to whether they were cocci or bacilli.

Behavioral study

Locomotion activity test

The locomotor activity test was performed in a square-shaped wooden arena ($30\times 80\times 80$ cm) with 64 squares painted on the arena floor (Ugo Basile, Italy). Several parameters were measured, including the number of squares crossed, washing, wall rearing, duration of locomotion, and immobility. Each mouse was placed in the experimental arena for 5 min under visual observations (*Ajarem & Ahmad, 1998*).

Shuttle box test

A shuttle box (Ugo Basile, Italy) was used to measure mouse avoidance reactions, which indicate mouse learning and memorizing abilities. The rectangular shuttle box was divided equally by a stainless-steel partition into two chambers with a gate providing access between the chambers. Each mouse was given 2 min with no stimulus to adapt to and familiarize itself with the shuttle box before beginning the trial sessions. A light bulb (21 W) and a buzzer (670 Hz and 70 dB) were turned on for 6 s consecutively as a conditioned stimulus (CS). After 5 s of CS, an unconditioned stimulus (US) that is electric scrambler shock (1 mA) was applied to the metallic grid floor for 4 s. The floor was a two-way procedure, so the shock (US) was delivered on either side of the metallic grid floor following the light and sound stimuli (CS). If the mouse avoided the US by escaping into the other chamber within 5 s after the CS, the microprocessor recorder unit of the shuttle box recorded an avoidance response. Each mouse underwent 30 trials with an intertrial interval of 15 s. The total number of avoidance responses and the total time taken to enter the other chamber to avoid the shock (latency of avoidance response or escape latency in seconds) were measured per mouse (*Abu-Taweel, Ajarem & Ahmad, 2014*).

Rotarod performance test

Here, a rotarod device (Ugo Basile, Italy) was used, with the mouse placed on a horizontally oriented rod that was mechanically rotating at 15 rpm. The rod is placed with at a low enough distance above the cage floor to avoid causing harm to the animal when they fall but high enough to encourage the animal to hold on and avoid falling. The amount of time an animal spends on this rotating rod is a measure of their physical ability, motor activity, balance, and coordination capability between the nervous and muscular systems (*Maodaa et al., 2016*).

Statistical analysis

Data were presented as the average ± standard deviation (SD). Using Dunnett's test for multiple comparisons, one-way analysis of variance tests was performed for all statistical comparisons between the studied groups. The Statistical Package for the Social Sciences (Chicago, IL, USA) was used to calculate the statistical analyses, and *p* values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Autism, as a neurodevelopmental condition, exhibits neuroinflammation in several brain areas. Activated astrocytes and microglia and elevated levels of proinflammatory cytokines demonstrate clear evidence of neuroinflammation in rodent models and individuals with autism (*Aabed et al., 2019*; *Nadeem et al., 2022*; *Kern et al., 2015*; *El-Ansary & Al-Ayadhi, 2012*).

Males and females show diverse functional correlations of neuroimmune signaling, which could be attributed to sexual dimorphism in cytokine activation in the brain (*Hudson, Jacobson-Pick & Anisman, 2014*). Generally, the female immune system includes numerous protective mechanisms that promote a stronger neuroimmune response when compared with that of the male immune system (*Fransen et al., 2017*).

In this study, females and males showed nonsignificant immune responses to PPA-induced neurotoxicity. Both sexes of PPA-treated mice showed approximately comparable IL-6 levels to those in the respective control groups (Table 1 and Fig. 2A). However, in contrast to the expected result, the level of IL-10 was significantly increased in both male ($2,939.40 \pm 361.78$ pg/ml) and female ($2,831.70 \pm 311.96$ pg/ml) mice (Table 1 and Fig. 2B). Studies by *Villa et al. (2015)* and *Abuaish et al. (2021a)* suggested that increased IL-10 levels in PPA-treated female rats act as protective mechanisms *via* the female sex hormone, estrogen, to activate the IL-10 promoter to support a significantly higher increase in IL-10 levels in females ($P < 0.001$) compared with a relatively lower elevation in males ($P < 0.017$). This could explain the resistance of female mice to developing biochemical and behavioral autistic characteristics after PPA exposure (*Abuaish et al., 2021a*). In addition, the anti-inflammatory effects of the physiological level of Glu *via* dendritic cell stimulation of T-reg cells to produce IL-10 could support the relationship between neurotransmission and immune response (*Van Sadelhoff et al., 2019*). The significant increase in IL-10 levels in PPA-treated male mice did not agree with the recent study by *Abuaish et al. (2021a)*, which demonstrated lower IL-10 levels in PPA-treated males. This could be attributed to

Table 1 Mean ± S.D of all the measured parameters in brain homogenate of PPA treated male and female mice groups compared to control group. Only *P* values ≤0.05 were considered significant.

Parameters	Groups	N	Min.	Max.	Mean ± S.D.	P value[a]	*P* value[b]
IL-6 (pg/ml)	Control Male	10	34.25	45.13	38.90 ± 3.41	0.786	0.688
	PPA Treated Male	6	21.27	86.11	42.99 ± 22.36		
	Control Female	9	20.21	43.03	34.50 ± 6.98	0.084	
	PPA Treated Female	6	30.49	48.76	41.29 ± 6.74		
IL-10 (pg/ml)	Control Male	10	2121.20	2712.10	2395.60 ± 140.33	0.017	0.593
	PPA Treated Male	6	2318.20	3444.40	2939.40 ± 361.78		
	Control Female	9	2186.90	2409.10	2287.90 ± 56.23	0.001	
	PPA Treated Female	6	2399.00	3368.80	2831.70 ± 311.96		
Glu (nmol/ml)	Control Male	10	17.68	22.00	20.09 ± 1.33	0.046	0.001
	PPA Treated Male	6	11.24	19.81	16.70 ± 3.15		
	Control Female	9	13.14	18.88	16.30 ± 1.93	0.001	
	PPA Treated Female	6	22.37	26.13	23.87 ± 1.71		
GABA (pg/ml)	Control Male	10	33.43	48.78	42.59 ± 4.30	0.026	0.123
	PPA Treated Male	6	43.63	52.77	47.62 ± 3.11		
	Control Female	9	45.34	48.50	46.95 ± 0.82	0.001	
	PPA Treated Female	6	48.62	51.41	49.84 ± 0.90		
LDH (U/ml)	Control Male	10	720.73	1182.17	1020.42 ± 126.37	0.021	0.094
	PPA Treated Male	6	522.97	1010.77	751.12 ± 204.27		
	Control Female	9	637.23	1164.59	852.58 ± 136.27	0.246	
	PPA Treated Female	6	652.61	1241.49	987.34 ± 236.88		
CK (IU/ml)	Control Male	10	582.29	1357.95	968.29 ± 261.51	0.660	0.337
	PPA Treated Male	6	430.68	2190.74	1063.88 ± 593.49		
	Control Female	9	465.83	1786.43	1027.49 ± 453.95	0.321	
	PPA Treated Female	6	509.78	1292.03	811.55 ± 283.89		
GSH (μg/ml)	Control Male	10	9.49	12.34	11.19 ± 0.88	0.307	0.873
	PPA Treated Male	6	6.01	14.24	9.81 ± 2.93		
	Control Female	9	7.91	14.24	10.64 ± 1.92	0.954	
	PPA Treated Female	6	6.96	17.72	10.55 ± 4.11		
GST (U/ml)	Control Male	10	0.23	0.37	0.31 ± 0.04	0.060	0.234
	PPA Treated Male	6	0.21	0.33	0.26 ± 0.04		
	Control Female	9	0.21	0.37	0.28 ± 0.06	0.545	
	PPA Treated Female	6	0.23	0.39	0.30 ± 0.05		
Catalase (U/ml)	Control Male	10	0.00	0.01	0.007 ± 0.002	0.012	0.225
	PPA Treated Male	6	0.01	0.02	0.012 ± 0.005		
	Control Female	9	0.00	0.01	0.008 ± 0.003	0.205	
	PPA Treated Female	6	0.01	0.01	0.010 ± 0.001		

Notes.
[a] *P* value between control and corresponding PPA traded group.
[b] *P* value between PPA treated groups.

the differences in rats and mice being used as rodent models of ASD, especially regarding social behavior and rewarding.

Several neuropsychiatric conditions, including ASD, have been recently connected to glutamatergic system dysfunction. Changes in the gut microbiota and Glu metabolism
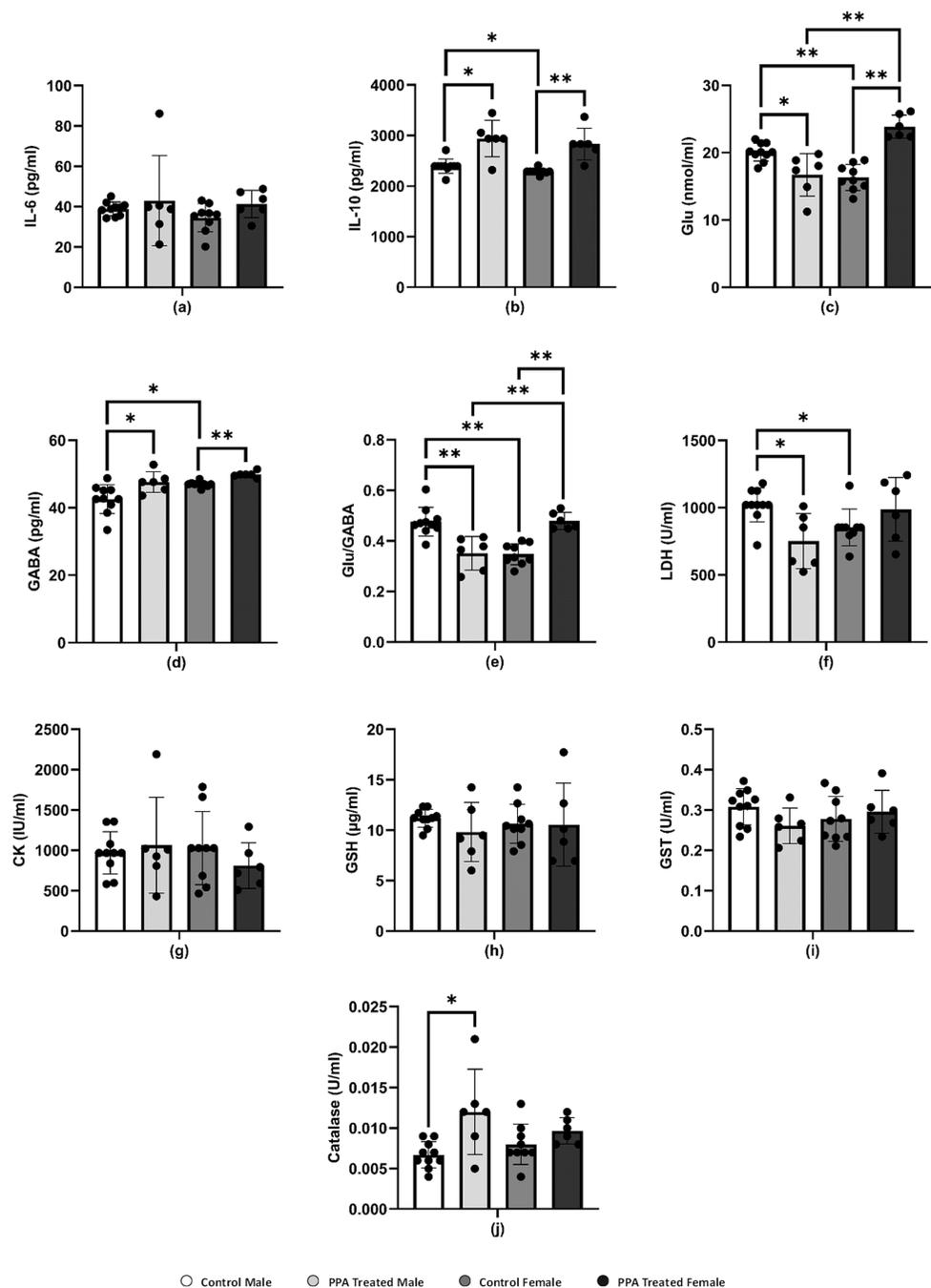
Figure 2 Comparing the levels of biochemical parameters in PPA-treated male and female mice to the comparable control groups. One asterisk (*), two asterisks (**), and three asterisks (***) show statistically significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively from the control group by *t*-test.

in patients with ASD provide evidence supporting the Glu-centered hypotheses of ASD as demonstrated in postmortem and patient samples and studies, which demonstrated abnormalities in glutamatergic gene expression and metabolic pathways (*Hollestein et*
al., 2023; *Montanari et al., 2022*). Over excitation of the Glu and glutamatergic receptors N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) acid activates enzymes that damage membrane permeability, electrochemical gradients, and cellular structure (*Essa et al., 2013*). Accordingly, several studies showed that people with ASD have lower levels of Glu metabolites in their basal ganglia and the anterior cingulate cortex and higher levels of Glu in their plasma (*Cai et al., 2016*; *Tebartz Van Elst et al., 2014*). Gln and Glu are related to each other in terms of structure and their immunomodulatory capabilities, and they are both necessary for intestinal growth and function. Lower plasma levels of Gln with concomitant higher levels of Glu have been reported in patients with ASD compared with those in healthy individuals. In this study, this was apparent with increased levels of Glu in PPA-treated female mice (Table 1 and Fig. 2C), which is not in good agreement with *Al-Suwailem, Abdi & El-Ansary (2018)*, who reported a significantly lower level of Glu in PPA-treated female rats compared with that in males. Despite this increase, this result does not contradict the lower vulnerability of female rats to develop biochemical autistic features as Glu-induced toxic effects only occur at much higher neuroexcitatory levels. This explanation can be supported by considering that control female rats had significantly less Glu than that in control males, which may be related to the significantly higher gene expression of Glu transporters in females that can prevent microglial Glu-induced neuronal death with PPA treatment (*Liang et al., 2008*; *Wickens, Bangasser & Briand, 2018*).

GABA is the most abundant inhibitory neurotransmitter in the brain (*Marshall, 2008*). γ-Aminobutyric acid type A (GABA$_A$) receptors, which are targeted by female sex hormones, are responsible for most inhibitory GABAergic effects in the brain (*Bäckström et al., 2014*). While estrogen reduces GABA inhibitory input, progesterone, and its neuroactive metabolites, allopregnanolone and pregnanolone, appear to increase the GABAergic inhibitory transmission *via* GABA$_A$ receptor activation (*Deligiannidis et al., 2013*). *Al-Suwailem, Abdi & El-Ansary (2018)* showed that female rats exhibited a significantly lower Glu/GABA ratio than that of male rats, which may be related to the lower susceptibility of females to the excitotoxic action of Glu *via* sex hormone-potentiated GABA inhibitory neurotransmission. By contrast, this study reported a significant increase in brain GABA in both male and female PPA-treated mice (Table 1 and Fig. 2D) with a remarkable higher Glu/GABA ratio in female treated rats (Fig. 2E), which again could be attributed to the difference in the rodents used. These species differences are extremely relevant in modeling social behavior deficits considering the collaborative and complex nature of human social behavior. Given their intrinsic lack of receptiveness to social interaction, mice may not be the ideal model of ASD, where reductions in social communication and social behavior are core symptoms (*Ellenbroek & Youn, 2016*; *Moy et al., 2008*).

LDH is an energy metabolism regulator found in the cytoplasm of many tissues, including the kidney, heart, and brain. Leakage of LDH from the brain to blood is a well-accepted neurotoxic phenomenon. Here, the LDH level increased in PPA-treated female mice but decreased in PPA-treated male mice, which displayed autism-like behavior (Table 1 and Fig. 2F). The significant decrease in brain LDH in PPA-treated male mice ($P < 0.021$) could be used as an index of susceptibility to PPA neurotoxic brain injury. By contrast,

the marked but nonsignificant increase in LDH levels in females may act as a protective strategy by increasing the glycolytic influx to compensate for ATP depletion as a marker of impaired energy metabolism (*Choi et al., 2018*; *Ippolito et al., 2017*; *Ross et al., 2010*).

In the brain, CK plays a critical role in maintaining ATP levels in neurons and is considered as a potential biomarker for diagnosing ASD since patients with autism suffer from mitochondrial dysfunction (*Khemakhem et al., 2017*). A nonsignificant elevation of CK was present in PPA-treated male mice compared with that in the control (Table 1 and Fig. 2G), whereas this declined in PPA-treated female mice. This result was supported by *El-Ansary, Bacha & Kotb (2012)* and can be explained by an increase in Ca^{2+}/Mg^{2+} and Na^+/K^+ ATPase activities as well as a decrease in the expression of mitochondrial electron transport chain complexes, which was demonstrated in many brain areas of children with autism compared with that in controls (*Chauhan et al., 2011*). *Al-Mosalem et al. (2009)* also noticed increased CK levels as well as reduced ATP levels in the plasma of Saudi patients with autism. This again could be attributed to the absence of ATP depletion in PPA-treated females through the much higher neuroprotective glycolytic influx previously discussed.

Oxidative stress is a condition that develops when ROS, including superoxide, H_2O_2, and OH radicals, accumulate in the cells or the antioxidant system fails (*Pizzino et al., 2017*). The brain is particularly vulnerable to oxidative stress because it contains high levels of lipids and consumes large amounts of energy as well as having limited antioxidant ability (*Rossignol & Frye, 2014*). GSH is the most powerful nonenzymatic antioxidant and serves a variety of roles (*Pizzino et al., 2017*), including protecting cells from free radicals, catalysis, metabolism, and transport and playing a role in immunity, cell proliferation, and apoptosis (*Lu, 2013*). Low GSH levels have been linked to neurological diseases such as Parkinson's disease, schizophrenia, and autism (*James et al., 2006*). This study showed a decrease in the level of GSH in both PPA-treated male and female groups compared with that in the control (Table 1 and Fig. 2H), and therefore the GSH depletion in the present study is in good agreement with results by *El-Ansary, Bacha & Kotb (2012)*.

Catalase and GST play a critical part in the antioxidant defense mechanisms by detoxifying xenobiotics and inactivating a wide range of endogenous oxidative stress products (*Mandic-Maravic et al., 2019*). In recent years, a significant reduction in GST activity has been observed in patients with ASD as compared with typically developed controls (*Mandic-Maravic et al., 2021*). Here, GST levels in PPA-treated male mice (0.26 ± 0.04 U/ml) were much lower than those in controls (0.31 ± 0.04 U/ml). However, these levels are considerably increased in females, which may assist in the detoxification of the neurotoxic dose of PPA (Table 1 and Fig. 2I). This could be related to females being less susceptible to autism as an acquired cellular detoxification deficiency syndrome (*James, 2018*). Furthermore, this result could be supported through the work of *Das et al. (1981)*, who reported much higher GST levels in female rats compared with those in males during postnatal development. Recently, *Abuaish et al. (2021a)* demonstrated that female rats exhibited a significant increase in levels of GST and GSH in response to PPA toxicity compared with those in PPA-treated male rats.

As an enzyme antioxidant system, catalase reduces the buildup of H_2O_2 in cells, especially in the brain (*Pizzino et al., 2017*). Schizophrenia and other illnesses have been related to

catalase deficiency or low activity (*Góth & Vitai, 1996*). Indeed, catalase is known to be effective in preventing neuronal degeneration (*Busciglio & Yankner, 1995*). The PPA-treated male and female mice in this study showed elevated catalase activity and this could be attributed to the harmful impact of PPA (Table 1 and Fig. 2J). An increase in catalase as a marker of oxidative stress was repeatedly reported in several studies (*Kalemci et al., 2017*; *Niu et al., 2018*). Collectively although differences in most of the measured variables make any biologically important difference between the neurochemistry of PPA-treated male and female mice difficult to prove, this study suggests that sex chromosomal gene dosage and sex hormone levels may play a role in determining sex-specific liability thresholds. However, considerably more research is needed to definitively identify the most important players and elucidate the precise mechanisms whereby these sex-specific factors modulate ASD phenotype presentation (*Werling & Geschwind, 2013*).

The change in the relative abundance of certain fecal bacteria strains in PPA-treated male and female mice compared with that in healthy controls is shown in Table 2. Although the abundance of *Staphylococcus* noticeably increased in both sexes, *Moraxell* a species were completely absent in the male cohort. Moreover, a marked difference was present in the abundance of gut bacteria of control males and females compared with respective PPA-treated groups showing much lower abundance of *Moraxella* species. This is in good agreement with the recent work of *Al Dera et al. (2021)*, which reported a decrease in the abundance of certain bacteria, including *Moraxella*, in individuals with autism and the PPA-rodent model of ASD (*Busciglio & Yankner, 1995*). Developing children are known to have a higher abundance of *Moraxella* than that in individuals with ASD, who had 31.9% reduced abundance of this bacterial species (*Forsyth et al., 2020*). In a mouse model of ASD fed a casein and gluten-rich diet, the abundance of fecal *Moraxella* was shown to be reduced concomitantly with a large increase in zonulin, a measure of gut leakiness as comorbidity in ASD (*Al Dera et al., 2021*). Lower abundance of *Moraxella* in the PPA-rodent model was attributed to the leaky gut as an accepted phenomenon relating gut microbiota to brain disorders *via* the GBA. This, the increasing trend of *Moraxella* abundance in treated female mice, and the complete absence in the male rodent model could help to suggest that female mice as a rodent model of autism had a lower tendency to develop leaky gut as a contributor of autistic features *via* the GBA. Moreover, the higher gram-positive/gram-negative bacterial ratio in females could be easily related to the resistance to developing gut leakiness compared with that in the male rodent model of ASD. Much higher levels of lipopolysaccharide as metabolites of gram-negative bacteria in males can easily pass the intestinal barrier and cause inflammation affecting the brain through altering cytokine levels (*Srikantha & Mohajeri, 2019*). This could demonstrate the role of sex in the marked susceptibility of males to developing autistic characteristics.

PPA administration is recognized as being able to impair social behavior and some cognitive tasks and induce convulsions and seizures, oxidative stress, and neuroinflammatory response in treated rodent brains (*Shultz et al., 2008*; *MacFabe, 2012*; *Shultz et al., 2009*). PPA-treated mice significantly crossed a greater number of squares on the locomotion monitor, indicating more horizontal distance was crossed compared with that by control mice (Table 3A). Similar to the horizontal distance result, the number

Table 2 Changes in bacteria growth in the feces of control and PPA treated male and female mice groups.

Group	Day	Staphylococus and/or Bacilli (Gram$^+$ cocci/rod or Gram$^-$ rod)	Enterobacteriacea (Gram$^-$ rod, lactose fermenters)	Gram$^+$/ Gram$^-$rod and cocci	Clostridium botulinum Gram$^+$, rod-shaped	Moraxella spp
Control Male	1	–	–	–	–	++++
	2	–	–	–	–	+++++
	3	–	–	–	–	+++++
PPA- Treated Male	1	+++	+	–	–	–
	2	++++	+	–	–	–
	3	++	+	–	–	–
Control Female	1	++	–	–	–	+++
	2	++	–	–	–	–
	3	+	–	–	–	++
PPA-Treated Female	1	++++	++++	++	–	++
	2	+++	–	++	–	+++
	3	+++	+++	++	–	+++

of vertical movements as wall rearing and center raring was also significantly increased in PPA-treated mice relative to controls. In addition, the total locomotion duration was significantly increased in PPA-treated mice, whereas immobility duration was markedly decreased in PPA-treated mice in both genders when compared with that in controls (Table 3A). Thus, the obtained data clearly indicates repetitive behavior and anxiety that represent core autistic-like behaviors in PPA-treated mice, which confirm the efficiency of the ASD mice model used. Similar findings were reported by *Rattanapornsompong et al. (2019)* and supported by *Sagheer et al. (2018)*, who reported that cerebellar neural bases for both social and motor abnormalities may be shared, and that motor dysfunction may contribute to social and communication deficiencies in ASDs. Similarly, cognitive skills are severely impaired in patients with ASD. Considerable efforts conducted by many studies have increased the understanding of the cognitive deficits in patients with ASD in different cognitive domains, such as the inability to attribute mental states, beliefs, intents, memory, planning, processing speed, and attention. In the current study, PPA-treated mice showed significantly more latency time for both genders compared with each corresponding control group, indicating the defect in learning and memorizing and thereby exposing the PPA-treated mice to harm (Table 3B and Fig. 3A). During light and sound stimulus both sexes of PPA-treated mice showed a smaller number of crossings compared with that in the control where they could comprehend the stimulus and run to avoid the shock; the decrease in crossings of PPA-treated male mice was significant, indicating a stronger cognitive defect (Table 3B and Fig. 3B). Similarly, PPA-treated female mice showed a significant increase in the number of reinforced crossings during the shock compared with that in the control, making them faster learners than PPA-treated male mice (Table 3B and Fig. 3C). The number of no crossings during the shock was significantly higher in PPA-treated male mice compared with that in the control, making males more susceptible

Table 3 Alterations in repetitive behavior, cognitive ability, and physical-neural coordination capability in PPA treated male and female mice groups compared to control (N = 6).

Indicators	Parameters	Groups	Mean ± S.D.	Significancy
		Control Male	207.47 ± 37.09	
	Number of Squares crossed	Control Female	167.17 ± 47.06	
		PPA Treated Male	265.67 ± 72.50	**
		PPA Treated Female	262.33 ± 65.23	*
		Control Male	19.17 ± 3.69	
	Wall rear	Control Female	17.00 ± 5.29	
		PPA Treated Male	27.00 ± 5.29	**
		PPA Treated Female	27.67 ± 7.64	*
A. Indicators of repetitive behavior and anxiety		Control Male	6.67 ± 7.02	
	Rear	Control Female	6.00 ± 5.57	
		PPA Treated Male	8.00 ± 5.00	*
		PPA Treated Female	8.00 ± 6.56	*
		Control Male	214.33 ± 48.69	
	Locomotion Duration (sec)	Control Female	193.33 ± 13.43	
		PPA Treated Male	282.00 ± 19.08	**
		PPA Treated Female	266.33 ± 14.29	*
		Control Male	88.67 ± 43.75	
	Immobility Duration (sec)	Control Female	70.67 ± 13.65	
		PPA Treated Male	24.67 ± 15.04	*
		PPA Treated Female	29.33 ± 16.80	*
		Control Male	110.17 ± 9.56	
	Total latency time (La)	Control Female	98.67 ± 5.57	
		PPA Treated Male	135.67 ± 5.09	**
		PPA Treated Female	122.50 ± 5.58	**
		Control Male	2.17 ± 0.75	
	Number of crossings during light and sound stimulus (St)	Control Female	1.83 ± 0.75	
		PPA Treated Male	0.50 ± 0.55	**
		PPA Treated Female	1.33 ± 0.52	
B. Indicators of cognitive ability		Control Male	22.00 ± 1.90	
	Number of reinforced crossings during the shock (Re)	Control Female	12.17 ± 1.94	
		PPA Treated Male	21.50 ± 5.58	
		PPA Treated Female	18.67 ± 2.88	**
		Control Male	2.33 ± 0.52	
	Number of no crossing during the shock (Tr)	Control Female	2.83 ± 0.75	
		PPA Treated Male	3.17 ± 0.75	*
		PPA Treated Female	3.67 ± 1.03	
		Control Male	3.67 ± 1.03	
C. Indicator of physical and coordination capability	Motor voltage measurement by wheels	Control Female	3.17 ± 0.75	
		PPA Treated Male	2.83 ± 0.75	
		PPA Treated Female	2.33 ± 0.52	*
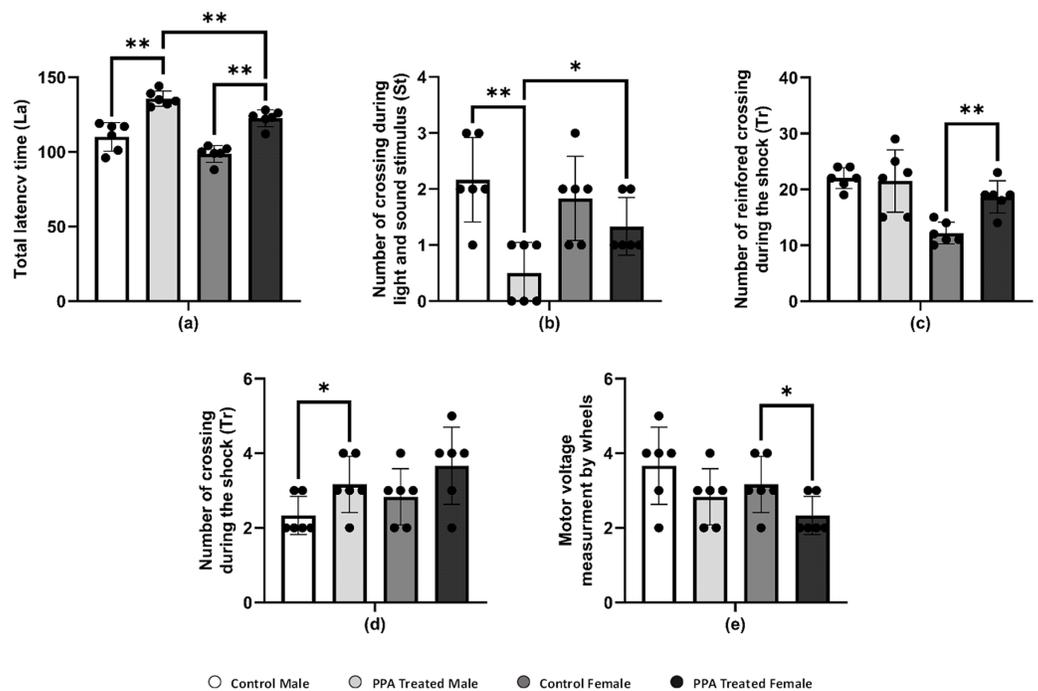
Figure 3 Alterations in repetitive behavior, cognitive ability, and physical–neural coordination capability in PPA treated male and female mice groups compared to control ($N = 6$). One asterisk (*), two asterisks (**), and three asterisks (***) show statistically significant differences at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively from the control group by t-test.

to be harmed by shocks then females were because of the higher degree of cognitive defects and decreased learning and memorizing in PPA-treated male mice (Table 3B and Fig. 3D). These findings are supported by a systematic review by *Velikonja, Fett & Velthorst (2019)*.

Moreover, there is an increasing belief that children with ASD exhibit motor impairments such as poor balance and postural control, incoordination, and unsteady gait (*Kaur & Bhat, 2019*). In this context, we performed a rotarod test in a mice model of ASD to investigate rodent motor coordination, which was particularly sensitive in detecting cerebellar dysfunction (*Shiotsuki et al., 2010*). PPA-treated male and female mice both exhibited a significant decrease in the duration of immobility, indicating motor impairments when compared with that in the control (Table 3C and Fig. 3E).

CONCLUSIONS

The biochemical findings of this study partially explain why male rats are more likely to develop biochemical and behavioral autistic features in response to a neurotoxic dose of PPA, although differences in the majority of the analyzed biomarkers make biologically relevant variation in the neurochemistry of PPA-treated male and female mice difficult to confirm. More research is required to definitively identify the most important contributors and the precise processes whereby these sex-specific factors influence ASD clinical presentation.

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The authors declare there are no competing interests.

Author Contributions
- Nasreen Kamalmaz performed the experiments, prepared figures and/or tables, and approved the final draft.
- Abir Ben Bacha conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Mona Alonazi conceived and designed the experiments, performed the experiments, prepared figures and/or tables, and approved the final draft.
- Gadah Albasher conceived and designed the experiments, performed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Arwa Ishaq A. Khayyat performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Afaf El-Ansary conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at the Faculty of Science-King Saud University (KSU-SE-19-92). Our study was carried out in compliance with the ARRIVE guidelines.

Data Availability
The raw measurements are available in the Supplemental Files.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.15488#supplemental-information.

REFERENCES

Aabed K, Bhat RS, Al-Dbass A, Moubayed N, Algahtani N, Merghani NM, Alanazi A, Zayed N, El-Ansary A. 2019. Bee pollen and propolis improve neuroinflammation and dysbiosis induced by propionic acid, a short chain fatty acid in a rodent model of autism. *Lipids in Health and Disease* **18(1)**:200 DOI 10.1186/s12944-019-1150-0.

Abu-Taweel GAZM, Ajarem JS, Ahmad M. 2014. Cognitive and biochemical effects of monosodium glutamate and aspartame, administered individually and in combination in male albino mice. *Neurotoxicology and Teratology* **42**:60–67 DOI 10.1016/j.ntt.2014.02.001.

Abuaish S, Al-Otaibi NM, Aabed K, Abujamel TS, Alzahrani SA, Alotaibi SM, Bhat RS, Arzoo S, El-Ansary A. 2021a. The role of sex-differentiated variations in stress hormones, antioxidants, and neuroimmune responses in relation to social interaction impairment in a rodent model of autism. *Metabolic Brain Disease* **36(6)**:1369–1379 DOI 10.1007/s11011-021-00732-5.

Abuaish S, Al-Otaibi NM, Abujamel TS, Alzahrani SA, Alotaibi SM, Alshawakir YA, Aabed K, El-Ansary A. 2021b. Fecal transplant and Bifidobacterium treatments modulate gut clostridium bacteria and rescue social impairment and hippocampal BDNF expression in a rodent model of autism. *Brain Sciences* **11(8)**:1038 DOI 10.3390/brainsci11081038.

Ajarem JS, Ahmad M. 1998. Prenatal nicotine exposure modifies behavior of mice through early development. *Pharmacology, Biochemistry, and Behavior* **59(2)**:313–318 DOI 10.1016/s0091-3057(97)00408-5.

Al Dera H, Alrafaei B, Al Tamimi MI, Alfawaz HA, Bhat RS, Soliman DA, Abuaish S, El-Ansary A. 2021. Leaky gut biomarkers in casein- and gluten-rich diet fed rat model of autism. *Translational Neuroscience* **12(1)**:601–610 DOI 10.1515/tnsci-2020-0207.

Al-Mosalem OA, El-Ansary A, Attas O, Al-Ayadhi L. 2009. Metabolic biomarkers related to energy metabolism in Saudi autistic children. *Clinical Biochemistry* **42(10–11)**:949–957 DOI 10.1016/j.clinbiochem.2009.04.006.

Al-Suwailem E, Abdi S, El-Ansary A. 2018. Sex differences in the glutamate signaling pathway in juvenile rats. *Journal of Neuroscience Research* **96(3)**:459–466 DOI 10.1002/jnr.24144.

Amador E, Dorfman LE, Wacker WE. 1963. Serum lactic dehydrogenase activity: an analytical assessment of current assays. *Clinical Chemistry* **12**:391–399.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. 2011. Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. *Brain, Behavior, and Immunity* **25(1)**:40–45 DOI 10.1016/j.bbi.2010.08.003.

Bäckström T, Bixo M, Johansson M, Nyberg S, Ossewaarde L, Ragagnin G, Savic I, Strömberg J, Timby E, Van Broekhoven F, Van Wingen G. 2014. Allopregnanolone and mood disorders. *Progress in Neurobiology* **113**:88–94 DOI 10.1016/j.pneurobio.2013.07.005.

Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, Kurzius-Spencer M, Zahorodny W, Rosenberg CR, White T, Durkin MS, Imm P, Nikolaou L, Yeargin-Allsopp M, Lee LC, Harrington R, Lopez M, Fitzgerald RT, Hewitt A, Dowling NF. 2018. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2014. Morbidity and Mortality Weekly Report. *Surveillance Summaries* **67(6)**:1–23 DOI 10.15585/mmwr.ss6706a1.

Bastiaanssen TFS, Cryan JF. 2021. The microbiota-gut-brain axis in mental health and medication response: parsing directionality and causality. *International Journal of Neuropsychopharmacology* **24(3)**:216–220 DOI 10.1093/ijnp/pyaa088.

Beutler E, Duron O, Kelly BM. 1963. Improved method for the determination of blood glutathione. *Journal of Laboratory and Clinical Medicine* **61**:882–888.

Bjørklund G, Meguid NA, El-Bana MA, Tinkov AA, Saad K, Dadar M, Hemimi M, Skalny AV, Hosnedlová B, Kizek R, Osredkar J, Urbina MA, Fabjan T, El-Houfey AA, Kałuzna-Czaplińska J, Gątarek P, Chirumbolo S. 2020. Oxidative stress in autism spectrum disorder. *Molecular Neurobiology* **57(5)**:2314–2332 DOI 10.1007/s12035-019-01742-2.

Bjørklund G, Saad K, Chirumbolo S, Kern JK, Geier DA, Geier MR, Urbina MA. 2016. Immune dysfunction and neuroinflammation in autism spectrum disorder. *Acta Neurobiologiae Experimentalis* **76(4)**:257–268 DOI 10.21307/ane-2017-025.

Bolnick DI, Snowberg LK, Hirsch PE, Lauber CL, Org E, Parks B, Lusis AJ, Knight R, Caporaso JG, Svanbäck R. 2014. Individual diet has sex-dependent effects on vertebrate gut microbiota. *Nature Communications* **5**:4500 DOI 10.1038/ncomms5500.

Borrás C, Sastre J, García-Sala D, Lloret A, Pallardó FV, Viña J. 2003. Mitochondria from females exhibit higher antioxidant gene expression and lower oxidative damage than males. *Free Radical Biology and Medicine* **34(5)**:546–552 DOI 10.1016/s0891-5849(02)01356-4.

Borrelli L, Aceto S, Agnisola C, De Paolo S, Dipineto L, Stilling RM, Dinan TG, Cryan JF, Menna LF, Fioretti A. 2016. Probiotic modulation of the microbiota-gut-brain axis and behaviour in zebrafish. *Scientific Reports* **6**:30046 DOI 10.1038/srep30046.

Bruce-Keller AJ, Keeling JL, Keller JN, Huang FF, Camondola S, Mattson MP. 2000. Antiinflammatory effects of estrogen on microglial activation. *Endocrinology* **141(10)**:3646–3656 DOI 10.1210/endo.141.10.7693.

Busciglio J, Yankner BA. 1995. Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. *Nature* **378(6559)**:776–779 DOI 10.1038/378776a0.

Cai J, Ding L, Zhang JS, Xue J, Wang LZ. 2016. Elevated plasma levels of glutamate in children with autism spectrum disorders. *NeuroReport* **27(4)**:272–276 DOI 10.1097/wnr.0000000000000532.

Chauhan A, Gu F, Essa MM, Wegiel J, Kaur K, Brown WT, Chauhan V. 2011. Brain region-specific deficit in mitochondrial electron transport chain complexes in children with autism. *Journal of Neurochemistry* **117(2)**:209–220 DOI 10.1111/j.1471-4159.2011.07189.x.

Choi J, Lee S, Won J, Jin Y, Hong Y, Hur TY, Kim JH, Lee SR, Hong Y. 2018. Pathophysiological and neurobehavioral characteristics of a propionic acid-mediated autism-like rat model. *PLOS ONE* **13(2)**:e0192925 DOI 10.1371/journal.pone.0192925.

Coretti L, Paparo L, Riccio MP, Amato F, Cuomo M, Natale A, Borrelli L, Corrado G, Comegna M, Buommino E, Castaldo G, Bravaccio C, Chiariotti L, Berni Canani RB, Lembo F. 2018. Gut microbiota features in young children with autism spectrum disorders. *Frontiers in Microbiology* **10**:920 DOI 10.3389/fmicb.2019.00920.

Das M, Dixit R, Seth PK, Mukhtar H. 1981. Glutathione-S-transferase activity in the brain: species, sex, regional, and age differences. *Journal of Neurochemistry* **36(4)**:1439–1442 DOI 10.1111/j.1471-4159.1981.tb00584.x.

Deligiannidis KM, Sikoglu EM, Shaffer SA, Frederick B, Svenson AE, Kopoyan A, Kosma CA, Rothschild AJ, Moore CM. 2013. GABAergic neuroactive steroids and resting-state functional connectivity in postpartum depression: a preliminary study. *Journal of Psychiatric Research* **47(6)**:816–828 DOI 10.1016/j.jpsychires.2013.02.010.

El-Ansary A, Al-Ayadhi L. 2012. Neuroinflammation in autism spectrum disorders. *Journal of Neuroinflammation* **9**:265 DOI 10.1186/1742-2094-9-265.

El-Ansary A, Al-Salem HS, Asma A, Al-Dbass A. 2017. Glutamate excitotoxicity induced by orally administered propionic acid, a short chain fatty acid can be ameliorated by bee pollen. *Lipids in Health and Disease* **16(1)**:1–9 DOI 10.1186/s12944-017-0485-7.

El-Ansary AK, Bacha AB, Kotb M. 2012. Etiology of autistic features: the persisting neurotoxic effects of propionic acid. *Journal of Neuroinflammation* **9(1)**:74 DOI 10.1186/1742-2094-9-74.

Ellenbroek B, Youn J. 2016. Rodent models in neuroscience research: is it a rat race? *Disease Models and Mechanisms* **9(10)**:1079–1087 DOI 10.1242/dmm.026120.

Engler-Chiurazzi EB, Singh M, Simpkins JW. 2016. From the 90′ to now: a brief historical perspective on more than two decades of estrogen neuroprotection. *Brain Research* **1633**:96–100 DOI 10.1016/j.brainres.2015.12.044.

Essa MM, Braidy N, Vijayan KR, Subash S, Guillemin GJ. 2013. Excitotoxicity in the pathogenesis of autism. *Neurotoxicity Research* **23(4)**:393–400 DOI 10.1007/s12640-012-9354-3.

Estes A, Zwaigenbaum L, Gu H, St John T, Paterson S, Elison JT, Hazlett H, Botteron K, Dager SR, Schultz RT, Kostopoulos P, Evans A, Dawson G, Eliason J, Alvarez S, Piven J, IBIS network. 2015. Behavioral, cognitive, and adaptive development in infants with autism spectrum disorder in the first 2 years of life. *Journal of Neurodevelopmental Disorders* **7(1)**:1–10 DOI 10.1186/s11689-015-9117-6.

Fattorusso A, Di Genova L, Dell'isola GB, Mencaroni E, Esposito S. 2019. Autism spectrum disorders and the gut microbiota. *Nutrients* **11(3)**:521 DOI 10.3390/nu11030521.

Ford TC, Nibbs R, Crewther DP. 2017. Glutamate/GABA+ ratio is associated with the psychosocial domain of autistic and schizotypal traits. *PLOS ONE* **12(7)**:e0181961 DOI 10.1371/journal.pone.0181961.

Forsyth A, Raslan K, Lyashenko C, Bona S, Snow M, Khor B, Herrman E, Ortiz S, Choi D, Maier T, Machida CA. 2020. Children with autism spectrum disorder: pilot studies examining the salivary microbiome and implications for

gut metabolism and social behavior. *Human Microbiome Journal* **15**:100066
DOI 10.1016/j.humic.2019.100066.

Fransen F, Van Beek AA, Borghuis T, Meijer B, Hugenholtz F, Van der Gaast-de Jongh
C, Savelkoul HF, De Jonge MI, Faas MM, Boekschoten MV, Smidt H, Aidy SEl,
De Vos P. 2017. The impact of gut microbiota on gender-specific differences in
immunity. *Frontiers in Immunology* **8**:754 DOI 10.3389/fimmu.2017.00754.

Goines PE, Ashwood P. 2013. Cytokine dysregulation in autism spectrum disorders
(ASD): possible role of the environment. *Neurotoxicology and Teratology* **36**:67–81
DOI 10.1016/j.ntt.2012.07.006.

Góth L, Vitai M. 1996. Hypocatalasemia in hospital patients. *Clinical Chemistry*
42(2):341–342 DOI 10.1093/clinchem/42.2.341.

Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione S-transferases. The first en-
zymatic step in mercapturic acid formation. *Journal of Biological Chemistry*
249(22):7130–7139 DOI 10.1016/S0021-9258(19)42083-8.

Herbert MR. 2011. SHANK3, the synapse, and autism. *New England Journal of Medicine*
365(2):173–175 DOI 10.1056/NEJMcibr1104261.

Holdeman LV, Moore WEC, Cato EP. 1977. *Anaerobe laboratory manual.* 4th edition.
Blacksburg: Anaerobe Laboratory, Virgina Polytechnic Institute and State University,
130.

Hollestein V, Poelmans G, Forde NJ, Beckmann CF, Ecker C, Mann C, Schäfer T,
Moessnang C, Baumeister S, Banaschewski T, Bourgeron T, Loth E, Dell'Acqua F,
Murphy DGM, Puts NA, Tillmann J, Charman T, Jones EJH, Mason L, Ambrosino
S, Holt R, Bölte S, Buitelaar JK, Naaijen J. 2023. Excitatory/inhibitory imbalance in
autism: the role of glutamate and GABA gene-sets in symptoms and cortical brain
structure. *Translational Psychiatry* **13(1)**:18 DOI 10.1038/s41398-023-02317-5.

Hu T, Dong Y, He C, Zhao M, He Q. 2020. The gut microbiota and oxidative stress
in autism spectrum disorders (ASD). *Oxidative Medicine and Cellular Longevity*
2020:8396708 DOI 10.1155/2020/8396708.

Hudson SP, Jacobson-Pick S, Anisman H. 2014. Sex differences in behavior and pro-
inflammatory cytokine mRNA expression following stressor exposure and re-
exposure. *Neuroscience* **277**:239–249 DOI 10.1016/j.neuroscience.2014.07.007.

Ippolito JE, Yim AK, Luo J, Chinnaiyan P, Rubin JB. 2017. Sexual dimorphism in
glioma glycolysis underlies sex differences in survival. *JCI Insight* **2(15)**:e92142
DOI 10.1172/jci.insight.92142.

Jack CR, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM,
Jagust W, Jessen F, Karlawish J, Liu E. 2018. NIA-AA research framework: toward a
biological definition of alzheimer's disease. *Alzheimer's and Dementia* **14(4)**:535–562
DOI 10.1016/j.neuropharm.2019.03.002.

James LW. 2018. Autism is an acquired cellular detoxification deficiency syn-
drome with heterogeneous genetic predisposition. *Autism-Open Access* **8**:1–25
DOI 10.4172/2165-7890.1000224.

James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH, Cutler P, Bock K,
Boris M, Bradstreet JJ, Baker SM, Gaylor DW. 2006. Metabolic endophenotype and

related genotypes are associated with oxidative stress in children with autism. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics* **141B**(**8**):947–956 DOI 10.1002/ajmg.b.30366.

Jia M, Njapo SAN, Rastogi V, Hedna VS. 2015. Taming glutamate excitotoxicity: strategic pathway modulation for neuroprotection. *CNS Drugs* **29**(**2**):153–162 DOI 10.1007/s40263-015-0225-3.

Kalemci O, Aydin HE, Kizmazoglu C, Kaya I, Yılmaz H, Arda NM. 2017. Effects of quercetin and mannitol on erythropoietin levels in rats following acute severe traumatic brain injury. *Journal of Korean Neurosurgical Society* **60**(**3**):355–361 DOI 10.3340/jkns.2016.0505.015.

Kaplan H, Hill K, Lancaster J, Hurtado AM. 2000. A theory of human life history evolution: diet, intelligence, and longevity. *Evolutionary Anthropology* **9**(**4**):156–185 DOI 10.1002/1520-6505(2000)9:4<156::AID-EVAN5>3.0.CO;2-7.

Kaur M, Bhat A. 2019. Creative yoga intervention improves motor and imitation skills of children with autism spectrum disorder. *Physical Therapy* **99**(**11**):1520–1534 DOI 10.1093/ptj/pzz115.

Kazdoba TM, Leach PT, Yang M, Silverman JL, Solomon M, Crawley J. 2016. Translational mouse models of autism: advancing toward pharmacological therapeutics. *Current Topics in Behavioral Neurosciences* **28**:1–52.

Kern JK, Geier DA, Sykes LK, Geier MR. 2015. Relevance of neuroinflammation and encephalitis in autism. *Frontiers in Cellular Neuroscience* **9**:519 DOI 10.3389/fncel.2015.00519.

Khemakhem AM, Frye RE, El-Ansary A, Al-Ayadhi L, Bacha AB. 2017. Novel biomarkers of metabolic dysfunction is autism spectrum disorder: potential for biological diagnostic markers. *Metabolic Brain Disease* **32**(**6**):1983–1997 DOI 10.1007/s11011-017-0085-2.

Kreiser NL, White SW. 2014. ASD in females: are we overstating the gender difference in diagnosis? *Clinical Child and Family Psychology Review* **17**(**1**):67–84 DOI 10.1007/s10567-013-0148-9.

Lebesgue D, Chevaleyre V, Zukin RS, Etgen AM. 2009. Estradiol rescues neurons from global ischemia-induced cell death: multiple cellular pathways of neuroprotection. *Steroids* **74**(**7**):555–561 DOI 10.1016/j.steroids.2009.01.003.

Li M, Liang P, Li Z, Wang Y, Zhang G, Gao H, Wen S, Tang L. 2015. Fecal microbiota transplantation and bacterial consortium transplantation have comparable effects on the re-establishment of mucosal barrier function in mice with intestinal dysbiosis. *Frontiers in Microbiology* **6**:692 DOI 10.3389/fmicb.2015.00692.

Liang J, Takeuchi H, Doi Y, Kawanokuchi J, Sonobe Y, Jin S, Yawata I, Li H, Yasuoka S, Mizuno T, Suzumura A. 2008. Excitatory amino acid transporter expression by astrocytes is neuroprotective against microglial excitotoxicity. *Brain Research* **1210**:11–19 DOI 10.1016/j.brainres.2008.03.012.

Lu SC. 2013. Glutathione synthesis. *Biochimica et Biophysica Acta* **1830**(**5**):3143–3153 DOI 10.1016/j.bbagen.2012.09.008.

MacFabe DF. 2012. Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. *Microbial Ecology in Health and Disease* **23(0)** DOI 10.3402/mehd.v23i0.19260.

MacFabe DF, Cain NE, Boon F, Ossenkopp KP, Cain DP. 2011. Effects of the enteric bacterial metabolic product propionic acid on object-directed behavior, social behavior, cognition, and neuroinflammation in adolescent rats: relevance to autism spectrum disorder. *Behavioural Brain Research* **217(1)**:47–54 DOI 10.1016/j.bbr.2010.10.005.

Maehly AC, Chance B. 1954. The assay of catalases and peroxidases. *Methods of Biochemical Analysis* **1**:357–424 DOI 10.1002/9780470110171.ch14.

Maenner MJ, Shaw KA, Baio J, Washington A, Patrick M, Di Rienzo M, Christensen DL, Wiggins LD, Pettygrove S, Andrews JG, Lopez M, Hudson A, Baroud T, Schwenk Y, White T, Rosenberg CR, Lee LC, Harrington RA, Huston M, Dietz PM. 2020. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2016. Morbidity and Mortality Weekly Report. *Surveillance Summaries* **69(4)**:1–12 DOI 10.15585/MMWR.SS6904A1.

Mandic-Maravic V, Coric V, Mitkovic-Voncina M, Djordjevic M, Savic-Radojevic A, Ercegovac M, Matic M, Simic T, Lecic-Tosevski D, Toskovic O, Pekmezovic T, Pljesa-Ercegovac M, Pejovic-Milovancevic M. 2019. Interaction of Glutathione S-transferase polymorphisms and tobacco smoking during pregnancy in susceptibility to autism spectrum disorders. *Scientific Reports* **9(1)**:3206 DOI 10.1038/s41598-019-39885-w.

Mandic-Maravic V, Mitkovic-Voncina M, Pljesa-Ercegovac M, Savic-Radojevic A, Djordjevic M, Ercegovac M, Pekmezovic T, Simic T, Pejovic-Milovancevic M. 2021. Glutathione S-transferase polymorphisms and clinical characteristics in autism spectrum disorders. *Frontiers in Psychiatry* **12(Jun)**:672389 DOI 10.3389/fpsyt.2021.672389.

Maodaa SN, Allam AA, Ajarem J, Abdel-Maksoud MA, Al-Basher GI, Wang ZY. 2016. Effect of parsley (Petroselinum crispum, Apiaceae) juice against cadmium neurotoxicity in albino mice (Mus musculus). *Behavioral and Brain Functions* **12(1)**:6 DOI 10.1186/s12993-016-0090-3.

Marshall FH. 2008. The role of GABA(B) receptors in the regulation of excitatory neurotransmission. *Results and Problems in Cell Differentiation* **44**:87–98 DOI 10.1007/400_2007_038.

Martin CR, Osadchiy V, Kalani A, Mayer EA. 2018. The brain-gut-microbiome axis. *Cellular and Molecular Gastroenterology and Hepatology* **6(2)**:133–148 DOI 10.1016/j.jcmgh.2018.04.003.

Montanari M, Martella G, Bonsi P, Meringolo M. 2022. Autism spectrum disorder: focus on glutamatergic neurotransmission. *International Journal of Molecular Sciences* **23(7)**:3861 DOI 10.3390/ijms23073861.

Morales T, Lorenson M, Walker AM, Ramos E. 2014. Both prolactin (PRL) and a molecular mimic of phosphorylated PRL, S179D-PRL, protect the hippocampus of female rats against excitotoxicity. *Neuroscience* **258**:211–217 DOI 10.1016/j.neuroscience.2013.11.015.

Moy SS, Nadler JJ, Young NB, Nonneman RJ, Segall SK, Andrade GM, Crawley JN, Magnuson TR. 2008. Social approach and repetitive behavior in eleven inbred mouse strains. *Behavioural Brain Research* **191(1)**:118–129 DOI 10.1016/j.bbr.2008.03.015.

Muscatello RA, Rafatjoo E, Mirpuri KK, Kim A, Vandekar S, Corbett BA. 2022. Salivary testosterone in male and female youth with and without autism spectrum disorder: considerations of development, sex, and diagnosis. *Molecular Autism* **13(1)**:37 DOI 10.1186/s13229-022-00515-4.

Nadeem A, Ahmad SF, Al-Harbi NO, Al-Ayadhi LY, Sarawi W, Attia SM, Bakheet SA, Alqarni SA, Ali N, AsSobeai HM. 2022. Imbalance in pro-inflammatory and anti-inflammatory cytokines milieu in B cells of children with autism. *Molecular Immunology* **141**:297–304 DOI 10.1016/j.molimm.2021.12.009.

Nadeem A, Ahmad SF, Attia SM, Al-Ayadhi LY, Al-Harbi NO, Bakheet SA. 2019. Dysregulated enzymatic antioxidant network in peripheral neutrophils and monocytes in children with autism. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **88**:352–359 DOI 10.1016/j.pnpbp.2018.08.020.

Nankova BB, Agarwal R, MacFabe DF, La Gamma EF. 2014. Enteric bacterial metabolites propionic and butyric acid modulate gene expression, including CREB-dependent catecholaminergic neurotransmission, in PC12 cells—possible relevance to autism spectrum disorders. *PLOS ONE* **9(8)**:e103740 DOI 10.1371/journal.pone.0103740.

Navarro F, Liu Y, Rhoads JM. 2016. Can probiotics benefit children with autism spectrum disorders? *World Journal of Gastroenterology* **22(46)**:10093–10102 DOI 10.3748/wjg.v22.i46.10093.

Niu X, Zheng S, Liu H, Li S. 2018. Protective effects of taurine against inflammation, apoptosis, and oxidative stress in brain injury. *Molecular Medicine Reports* **18(5)**:4516–4522 DOI 10.3892/mmr.2018.9465.

Pangrazzi L, Balasco L, Bozzi Y. 2020. Oxidative stress and immune system dysfunction in autism spectrum disorders. *International Journal of Molecular Sciences* **21(9)**:3293 DOI 10.3390/ijms21093293.

Parkin GM, Udawela M, Gibbons A, Dean B. 2018. Glutamate transporters, EAAT1 and EAAT2, are potentially important in the pathophysiology and treatment of schizophrenia and affective disorders. *World Journal of Psychiatry* **8(2)**:51–63 DOI 10.5498/wjp.v8.i2.51.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, Squadrito F, Altavilla D, Bitto A. 2017. Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity* **2017**:8416763 DOI 10.1155/2017/8416763.

Prokai-Tatrai K, Prokai L, Simpkins JW, Jung ME. 2009. Phenolic compounds protect cultured hippocampal neurons against ethanol-withdrawal induced

oxidative stress. *International Journal of Molecular Sciences* **10**(**4**):1773–1787 DOI 10.3390/ijms10041773.

Qasem H, Al-Ayadhi L, El-Ansary A. 2016. Cysteinyl leukotriene correlated with 8-isoprostane levels as predictive biomarkers for sensory dysfunction in autism. *Lipids in Health and Disease* **15**(**1**):130 DOI 10.1186/s12944-016-0298-0.

Rattanapornsompong K, Ngamkham J, Chavalit T, Jitrapakdee S. 2019. Generation of human pyruvate carboxylase knockout cell lines using retrovirus expressing short hairpin RNA and CRISPR-Cas9 as models to study its metabolic role in cancer research. *Methods in Molecular Biology* **1916**:273–288 DOI 10.1007/978-1-4939-8994-2_26.

Ristori MV, Quagliariello A, Reddel S, Ianiro G, Vicari S, Gasbarrini A, Putignani L. 2019. Autism, gastrointestinal symptoms and modulation of gut microbiota by nutritional interventions. *Nutrients* **11**(**11**):2812 DOI 10.3390/nu11112812.

Roman P, Rueda-Ruzafa L, Cardona D, Cortes-Rodríguez A. 2018. Gut-brain axis in the executive function of austism spectrum disorder. *Behavioural Pharmacology* **29**(**7**):654–663 DOI 10.1097/fbp.0000000000000428.

Rose S, Bennuri SC, Wynne R, Melnyk S, James SJ, Frye RE. 2017. Mitochondrial & redox abnormalities in autism lymphoblastoid cells: a sibling control study. *FASEB Journal* **31**(**3**):904–909 DOI 10.1096/fj.201601004r.

Ross JM, Öberg J, Brené S, Coppotelli G, Terzioglu M, Pernold K, Goiny M, Sitnikov R, Kehr J, Trifunovic A, Larsson NG, Hoffer BJ, Olson L. 2010. High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio. *Proceedings of the National Academy of Sciences of the United States of America* **107**(**46**):20087–20092 DOI 10.1073/pnas.1008189107.

Rossignol DA, Frye RE. 2014. Evidence linking oxidative stress, mitochondrial dysfunction, and inflammation in the brain of individuals with autism. *Frontiers in Physiology* **5**:150 DOI 10.3389/fphys.2014.00150.

Sagheer TAl, Haida O, Balbous A, Francheteau M, Matas E, Fernagut PO, Jaber M. 2018. Motor impairments correlate with social deficits and restricted neuronal loss in an environmental model of autism. *International Journal of Neuropsychopharmacology* **21**(**9**):871–882 DOI 10.1093/ijnp/pyy043.

Schnorr SL, Candela M, Rampelli S, Centanni M, Consolandi C, Basaglia G, Turroni S, Biagi E, Peano C, Severgnini M, Fiori J, Gotti R, De Bellis G, Luiselli D, Brigidi P, Mabulla A, Marlowe F, Henry AG, Crittenden AN. 2014. Gut microbiome of the Hadza hunter-gatherers. *Nature Communications* **5**:3654 DOI 10.1038/ncomms4654.

Sealey LA, Hughes BW, Sriskanda AN, Guest JR, Gibson AD, Johnson-Williams L, Pace DG, Bagasra O. 2016. Environmental factors in the development of autism spectrum disorders. *Environment International* **88**:288–298 DOI 10.1016/j.envint.2015.12.021.

Shiotsuki H, Yoshimi K, Shimo Y, Funayama M, Takamatsu Y, Ikeda K, Takahashi R, Kitazawa S, Hattori N. 2010. A rotarod test for evaluation of motor skill learning. *Journal of Neuroscience Methods* **189**(**2**):180–185 DOI 10.1016/j.jneumeth.2010.03.026.

Shultz SR, MacFabe DF, Martin S, Jackson J, Taylor R, Boon F, Ossenkopp KP, Cain DP. 2009. Intracerebroventricular injections of the enteric bacterial metabolic

product propionic acid impair cognition and sensorimotor ability in the Long-Evans rat: further development of a rodent model of autism. *Behavioural Brain Research* **200(1)**:33–41 DOI 10.1016/j.bbr.2008.12.023.

Shultz SR, MacFabe DF, Ossenkopp KP, Scratch S, Whelan J, Taylor R, Cain DP. 2008. Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: implications for an animal model of autism. *Neuropharmacology* **54(6)**:901–911 DOI 10.1016/j.neuropharm.2008.01.013.

Silva GA, Theriault E, Mills LR, Pennefather PS, Feeney CJ. 1999. Group I and II metabotropic glutamate receptor expression in cultured rat spinal cord astrocytes. *Neuroscience Letters* **263(2–3)**:117–120 DOI 10.1016/s0304-3940(99)00145-7.

Simpkins JW, Yi KD, Yang SH, Dykens JA. 2010. Mitochondrial mechanisms of estrogen neuroprotection. *Biochimica et Biophysica Acta* **1800(10)**:1113–1120 DOI 10.1016/j.bbagen.2009.11.013.

Srikantha P, Mohajeri MH. 2019. The possible role of the microbiota-gut-brain-axis in autism spectrum disorder. *International Journal of Molecular Sciences* **20(9)**:2115 DOI 10.3390/ijms20092115.

Szasz G, Gruber W, Bernt E. 1976. Creatine kinase in serum: 1. Determination of optimum reaction conditions. *Clinical Chemistry* **22(5)**:650–656 DOI 10.1093/clinchem/22.5.650.

Tebartz Van Elst L, Maier S, Fangmeier T, Endres D, Mueller GT, Nickel K, Ebert D, Lange T, Hennig J, Biscaldi M, Riedel A, Perlov E. 2014. Disturbed cingulate glutamate metabolism in adults with high-functioning autism spectrum disorder: evidence in support of the excitatory/inhibitory imbalance hypothesis. *Molecular Psychiatry* **19(12)**:1314–1325 DOI 10.1038/mp.2014.62.

Tejadilla D, Cerbón M, Morales T. 2010. Prolactin reduces the damaging effects of excitotoxicity in the dorsal hippocampus of the female rat independently of ovarian hormones. *Neuroscience* **169(3)**:1178–1185 DOI 10.1016/j.neuroscience.2010.05.074.

Tu WJ, Chen H, He J. 2012. Application of LC-MS/MS analysis of plasma amino acids profiles in children with autism. *Journal of Clinical Biochemistry and Nutrition* **51(3)**:248–249 DOI 10.3164/jcbn.12-45.

Van Sadelhoff JHJ, Pardo PP, Wu J, Garssen J, Van Bergenhenegouwen J, Hogenkamp A, Hartog A, Kraneveld AD. 2019. The gut-immune-brain axis in autism spectrum disorders; a focus on amino acids. *Frontiers in Endocrinology* **10**:1–15 DOI 10.3389/fendo.2019.00247.

Velikonja T, Fett AK, Velthorst E. 2019. Patterns of nonsocial and social cognitive functioning in adults with autism spectrum disorder: a systematic review and meta-analysis. *JAMA Psychiatry* **76(2)**:135–151 DOI 10.1001/jamapsychiatry.2018.3645.

Villa A, Rizzi N, Vegeto E, Ciana P, Maggi A. 2015. Estrogen accelerates the resolution of inflammation in macrophagic cells. *Scientific Reports* **5**:15224 DOI 10.1038/srep15224.

Wang Y, Zhao S, Liu X, Zheng Y, Li L, Meng S. 2018. Oxytocin improves animal behaviors and ameliorates oxidative stress and inflammation in autistic mice. *Biomedicine and Pharmacotherapy* **107**:262–269 DOI 10.1016/j.biopha.2018.07.148.

Werling DM, Geschwind DH. 2013. Sex differences in autism spectrum disorders. *Current Opinion in Neurology* **26(2)**:146–153 DOI 10.1097/wco.0b013e32835ee548.

Wickens MM, Bangasser DA, Briand LA. 2018. Sex differences in psychiatric disease: a focus on the glutamate system. *Frontiers in Molecular Neuroscience* **11**:197 DOI 10.3389/fnmol.2018.00197.

Zurita MF, Cárdenas PA, Sandoval ME, Peña MC, Fornasini M, Flores N, Monaco MH, Berding K, Donovan SM, Kuntz T, Gilbert JA, Baldeón ME. 2020. Analysis of gut microbiome, nutrition and immune status in autism spectrum disorder: a case-control study in Ecuador. *Gut Microbes* **11(3)**:453–464 DOI 10.1080/19490976.2019.1662260.