

REVIEW ARTICLE

Enteric Ecosystem Disruption in Autism Spectrum Disorder: Can the Microbiota and Macrobiota be Restored?

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Abstract: Background: Many lines of scientific research suggest that Autism Spectrum Disorders (ASDs) may be associated with alterations in the enteric ecosystem, including alterations of the enteric macrobiome (i.e. helminthes and fungi) and changes in predominant microbiome species, particularly a reduction in microbiome diversity.

Methods: We performed a comprehensive review of the literature and summarized the major findings.

Results: These alterations are believed to be due to a variety of factors including changes in the post-industrial society related to decreased exposure to symbiotic organisms, increased human migration, overuse of antibiotics and changes in dietary habits. Changes in the enteric ecosystem are believed to alter metabolic and immune system function and epigenetic regulation. If these changes occur during critical developmental windows, the trajectory of brain development, as well as brain function, can be altered. This paper reviews theoretical models that explain how these perturbations may in isolation or in combination be causative for ASDs as well as the preclinical and clinical studies that support these models. We discuss how these alterations may converge to trigger or exacerbate the formation of an ASD phenotype. We focus on possible preconception, prenatal, perinatal and postnatal factors that may alter the enteric ecosystem leading to physiological disruptions, potentially through triggering events.

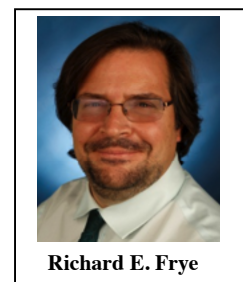
Conclusion: If these theoretical models prove to be valid, they may lead to the development of practical interventions which could decrease ASD prevalence and/or morbidity.

Keywords: Autism Spectrum Disorders, Microbiome, Macrobiome, Fauna, Helminths, Mitochondria, Cell Danger Theory, Biome Depletion, Propionic Acid, Oxidative Stress, Microbiota, Antibiotics, Treatment, Immune Function, Acetaminophen, Beta lactam, Omeprazole, Epigenetics.

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INTRODUCTION

Autism Spectrum Disorder (ASDs) is a heterogeneous neurodevelopmental disorder for which the etiology and pathophysiology continues to remain poorly understood. While chromosomal abnormalities and single gene defects account for a minority of ASD cases [1], for the most part, there is no single genetic abnormality that is causative for ASD. Indeed, there may be many synergistic heterozygous mutations that may be additive in nature to increase the risk of developing ASD [2]. Emerging research points to polygenic susceptibility interacting with environmental triggers at critical time points during prenatal, perinatal, and/or postnatal life that may alter key physiological systems to disrupt metabolism, brain development, and increase the likelihood of developing an ASD [3-7].

While genetic susceptibility may be a key contributor to ASDs, it may conceptually just "load the gun" so to speak, with prenatal, perinatal, and/or postnatal environmental exposures being the events that "pull the trigger" that give rise to the ASD phenotypes. Many lines of research point to the importance of the development of the microbiome during the first 3 years of life as being critical in the establishment of a healthy immune system, with intestinal

dysbiosis and inflammation being key driving forces behind many seemingly disparate disease processes.

The human body houses a diverse ecosystem of microbes, primarily bacteria, which inhabit niches in the body such as the gut, skin, lung, oral cavity, vagina, and placenta; these microbes and their genome are collectively referred to as the microbiome. The microbiome is tightly linked with host health and is an important contributor to host metabolism and immune system function. Interestingly, the human body houses 10 times more microbial cells than human cells with their genetic material outnumbering human DNA by 100-150 to 1 [8-10]. The microbiome has been referred to as the "forgotten organ" and numerous lines of research have implicated disruptions in the host microbiota with human disease [11]. The particular focus in human disease has been on the disruption of the enteric (gut) microbiota due to its relationship to metabolic and immune function, as well as vitamin creation, particularly since it accounts for approximately 99% of the human microbiome [12].

Disruptions in the microbiome can lead to a wide array of disease processes that affect the gut such as Inflammatory Bowel Diseases as well as conditions which are regarded as "autoimmune", such as Type I Diabetes, rheumatoid arthritis, and multiple sclerosis, just to name a few [13, 14]. Furthermore, an appreciation for the so called "microbiome-gut-brain" axis is gaining increased attention due to tri-directional feedback between the microbiome, gut and brain that can affect brain function and behavior, and poten-

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tially result in neurodevelopmental and neurodegenerative disease conditions [14-16].

The human macrobiome refers to the multicellular organisms that inhabit the intestinal milieu. It is believed that the microbiota (i.e. helminthes and fauna) which were symbionts that helped modulate and regulate the human immune system prior to the industrial revolution have largely been depleted from industrialized nations (see Biome Depletion Theory in this review). In this review we will focus on how elimination of these keystone microbiota species are just as important as the disrupted microbiota and represent another key element in intestinal ecosystem disruption in ASDs.

Emerging research has pointed to an important role of alterations in the microbiome and macrobiome in the development of disease, including neurodevelopmental disorders such as ASD. This review will focus on the emerging role of the enteric microbiome and macrobiome in the etiology and/or pathophysiology of ASD. Significantly, the prevalence of gastrointestinal (GI) symptoms in children with ASD ranges from 9% to 91%, so much research has focused on the GI connection to ASD. Despite this wide variation, it is becoming well accepted that GI symptoms are common in children with ASD [17-19]. Due to the recognition of GI disturbances in ASD, recent work has started to investigate the possibility that microbiome and macrobiome disruption during critical developmental windows could increase risk for developing an ASD and could explain the link between ASD and GI symptoms [20-24].

To investigate the possibility that enteric microbiome disruptions could potentiate ASD-like behavior, Hsiao et al. conducted a landmark mouse model trial that demonstrated that altering the enteric microbiome using a probiotic treatment (i.e. *B. fragilis*) can significantly attenuate ASD-like behaviors [25]. Interestingly, the authors noted that the mice with ASD-like symptoms had markedly elevated levels of the phenolic derivative 4-ethylphenylsulfate (4EPS) at 46 times higher concentration than the control cohort mice. The chemical properties of 4EPS are structurally similar to the analogous compound found in humans, p-cresol (4-methylphenol), which alters cellular membrane permeability, redox activity and ion channels, and has been shown to be elevated in urine from individuals with ASD [26-30].

Further, clinical evidence that the microbiome is altered in some children with ASD is demonstrated by decreased species diversity and overrepresentation of certain species such as *C. difficile* [31, 32]. Microbiome studies have suggested that children with regressive-type ASD may have particular abnormalities in their gut microbiome [33], and that children with overrepresentation or underrepresentation of particular bacterial species (i.e. *lactobacillus* sp.) may constitute a subgroup of children with ASD who present with GI symptoms at the time of or prior to the onset of ASD symptoms [34].

In a pivotal study, Sandler et al. focused on the theory that alterations in the microbiome may be related to ASD symptoms. In a small clinical trial, individuals with regressive-type ASD were treated with oral vancomycin. Since vancomycin is not absorbed and does not enter the systemic circulation, its effects are confined to the gut. The authors showed that oral vancomycin did in fact provide short term improvements in ASD symptoms, although the authors caution that the protocol is not intended for long term use, due to the possibility of the development of vancomycin resistant enterococcus with vancomycin overuse. Rather, this trial was used as a tool to further demonstrate the influence of the microbiome (and its manipulation) on ASD symptoms [34].

To this end we first briefly review some of the evidence for the role of the enteric microbiome in ASD. It is extremely important to keep in mind that while perturbations in the microbiome are very important, it is also the lack of exposure to helminthes and fauna in post-industrial societies that might be driving the high incidence of

inflammatory diseases. We then describe three primary theories related to ASD and the enteric ecosystem: the Biome Depletion Theory (BDT) [35], the Propionic Acid (PPA) model of ASD [36] and the Cell Danger Response (CDR) Theory [37]. Using these theories, as examples, we will discuss how changes in the microbiota and/or macrobiota or their metabolic end products may converge to disrupt key physiological systems, leading to abnormal redox and mitochondrial metabolism, as well as atypical development of neuronal circuitry and the immune system. Alterations in physiological systems can lead to secondary alterations in epigenetic regulation and/or *de novo* genetic mutations, thereby promoting aberrant neurodevelopment. We will discuss potential triggers which could, independently or collectively, lead to a complex cascade of physiological events that could increase risk for developing ASD or ASD-associated behaviors. Finally, we will cover what future directions may hold in terms possible preventative strategies that might lead to decreased disease prevalence.

THEORETICAL MODELS OF ENTERIC ECOSYSTEM DISRUPTION IN ASD

Biome Depletion Theory (BDT)

The Biome Depletion Theory (BDT), also referred to as the “lost friends theory,” sets forth the notion that practices that arose with post-industrial societies have dramatically altered our biome [38]. According to the BDT these changes, which include modern sanitation, water treatment, usage of toilets, modern medicine, agriculture, soaps, etc., have dramatically altered the human enteric ecosystem. The BDT suggests that disruption of the enteric macrobiome (i.e., multicellular organisms such as helminthes, protozoans and other fauna) is the key drivers of autoimmune and inflammatory processes, rather than microbiome disruption. The industrial practices and technological advances listed above are believed to have led to the elimination of the macrobiome organisms which co-evolved with humans over centuries, and, through natural selection, contributed to the stabilization and support of our immune system. This theory proposes that eradication of macrobiome organisms has led to a dramatic increase in hyperimmune associated allergic and autoimmune disease. Thus, “biome depletion” refers to the loss of macrobiome organisms from the biome, which in turn has led to decreased exposure to these organisms in post-industrial societies and that their loss has led to immune de-stabilization. Furthermore, there is evidence that helminthes co-evolved with the microbiota to provide modulatory support to the immune system and that host-microbe-helminth interactions are key factors in immune system regulatory networks [39-42].

The BDT grew out of the human hygiene hypothesis [43-45] which postulates that increased susceptibility to allergic disease is caused by a lack of exposure to symbiotic microorganisms, infectious agents, and parasites by virtue of our modern “cleanliness”. Bilbo et al. [46] postulate that biome depletion, in and of itself, may not necessarily be causative for disease, but when added to the mix of other factors such as westernized low vegetable fiber pro-inflammatory diets, sedentary lifestyles, chronic psychological stress, and/or Vitamin D deficiency, may, in combination, alter the immune system towards a hyperreactive and/or sensitive state (See Figure 1). This could, in turn, increase the likelihood of disease. Furthermore, Bilbo et al [46] have used an analogy of the ecosystem of the human body as a three-legged stool in which the macrobiome is one leg, the immune system is another, and the microbiome is the third leg. Fauna and protozoans, as constituents of the macrobiome, are not placed in the microbiome because of their mutual and similar effects on the immune system and because they are not considered to be a part of the modern microbiome since modern practices of industrialized nations have made them extinct from the intestinal ecosystem of most western societies. If there is disruption in any of these three areas, the ecosystem becomes de-

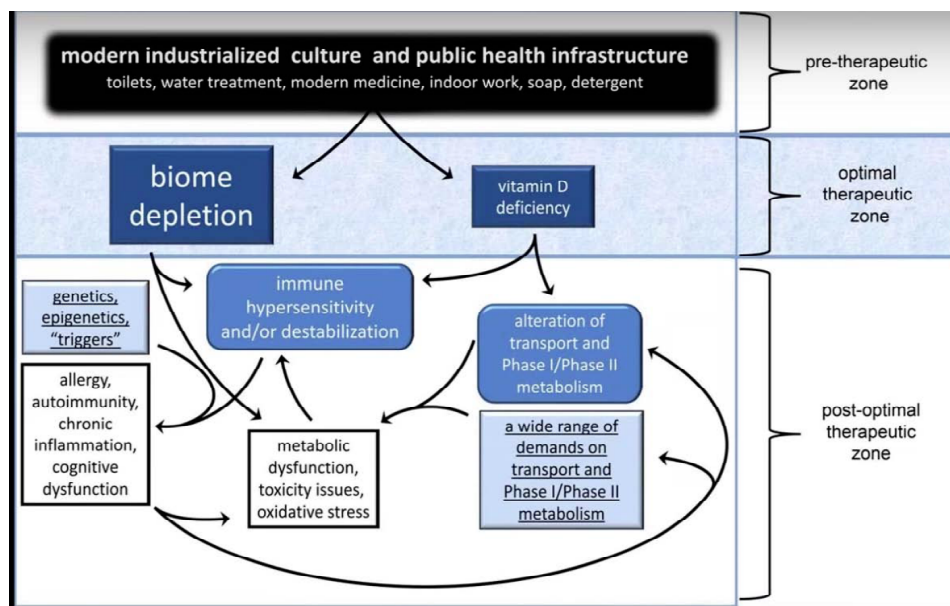


Fig. (1). Pre-therapeutic, optimal therapeutic and post-therapeutic windows that lead to physiological cascades that increase risk for cognitive dysfunction via immune-mediated and pro-inflammatory events. Other factors not listed that may contribute to this flow diagram include modern medicine and agriculture. Contributed by Dr William Parker.

stabilized and can lead to proinflammatory, allergic, and/or autoimmune related conditions.

BDT hypothesizes that alterations of the microbiome are a consequence of eradication of helminthes, protozoans and other symbionts that co-evolved with humans to regulate, stabilize, and provide modulatory activity for our immune system; their loss is what is hypothesized to drive the disease processes. Conceptually, the microbiome works with the other legs of the stool to promote stability of the human body’s ecosystem and immune function. The BDT hypothesizes that reconstitution of the human biome via reintroduction of helminthes or other macrobiome constituents is a reasonable and necessary step in order to ward off allergic, autoimmune, and inflammatory disease processes.

Bilbo et al. [38] postulate that ASD may fall into a larger family of hyperimmune-mediated disorders that are a consequence of post-industrial society. In this sense, this may be considered a modern pandemic that may be related, in part, due to biome depletion and associated factors. They propose a model in which ASD may be a result of a “three hit paradigm” (See Fig. 2).

The BDT is consistent with the “three hit paradigm”:

- Hit 1: Biome depletion.
- Hit 2: Environmental stimulus (e.g. acetaminophen exposure, vitamin D deficiency, antibiotic exposure, etc.) at critical times in development [47, 48].
- Hit 3: Genetic and/or epigenetic predisposition.

Bilbo et al [38] suggest that this theory can be tested using simple steps. The idea is to determine if inflammatory and/or cognitive diseases of childhood decline in response to steps which are aimed at enriching the biome. Examples of potential efforts at “biome reconstitution” include probiotics, microbiome transplantations, helminth reintroduction to the human ecosystem [49, 50], and/or minimizing the effects of chronic psychological stress, avoiding inflammatory diets, and decreasing sedentary lifestyles.

Propionic Acid Theory

The propionic acid (PPA) theory of ASD [51-53] suggests that ASDs may be a result of disturbances in the enteric microbiome

resulting in the increased production of the short chain fatty acid (SCFA) known as PPA. Microbes which have been reported to be in abundance in certain subgroups of ASD patient cohorts include *Clostridia*, *Bacteroides*, and *Desulfovibrio* species [54]. PPA as well as other SCFAs can alter diverse metabolic and immune pathways, gene expression and synaptic plasticity, in a manner that is consistent with findings of ASD. Particularly interesting is upregulation of cyclic adenosine monophosphate response element binding protein (CREB), which plays a major regulatory role in synaptic plasticity and the neurobiology of seizures, movement, as well as reward systems and memory [55]. MacFabe [3, 36] has proposed that phosphorylation dependent upregulation of CREB activity results in enhancement of memory, causing in an “inability to forget” which could explain anxiety, perseverative, and repetitive behaviors which are partially diagnostic for ASD.

MacFabe and colleagues [36, 50, 56] concentrated on PPA as an important enteric SCFA increased in ASD stool, and produced directly or indirectly by many ASD associated bacteria (i.e. *clostridial* & *disulfovibrio* species), following the fermentation of refined and wheat based sugars. Furthermore, PPA is a ubiquitous molecule that naturally occurs in many food products (wheat and dairy), and is also added to many refined foods as a preservative. In addition, it is an important intermediate of fatty acid energy metabolism in humans [57], and is known to be associated with particular inborn metabolic errors of metabolism, many of which are underreported (propionic/methylmalonic acidurias, holocarboxylase deficiency, biotinidase deficiency), and show some clinical similarities to ASD [58]. PPA has broad effects in brain, gut and immune systems, including specific activation of free fatty acid G coupled receptors, neurotransmitter synthesis and release, innate immunity, mitochondrial/lipid function, gap/tight junctional gating, and alteration of gene expression, all for which have been linked to ASD [50]. Further, since PPA is a small, lipid-soluble molecule, it can easily pass across membranes by both passive and active means (i.e. monocarboxylate receptors). It is also rapidly metabolized. Thus, it could have both acute and transient effects on metabolic systems throughout the lifecycle, which could account for clinical reports of waxing and waning of behavior in children with ASD

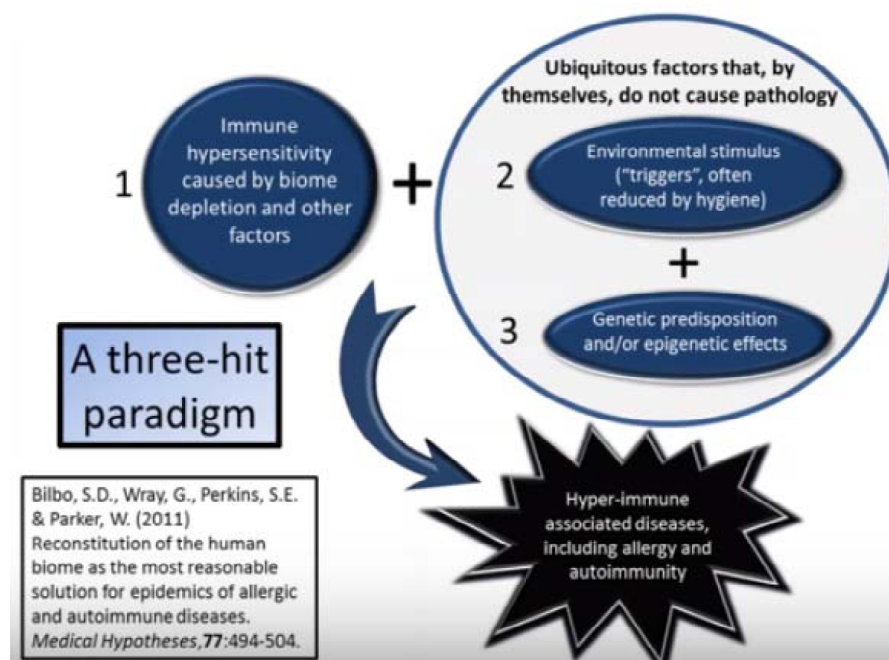


Fig. (2). The Three Hit Paradigm. From Bilbo *et al.* [38] with permission.

after consumption of certain food products rich in refined dietary carbohydrates.

To test the central effect of PPA, MacFabe *et al.* [57] infused PPA, related SCFAs (butyrate, acetate) and other control compounds (propanol) into the central nervous system (CNS) of adult rats via brief intracerebroventricular (ICV) pulses. ICV PPA infusions resulted in reversible ASD-like behaviors (repetitive, impaired social, object preference, perseveration) as well as electrographic and metabolic changes (innate neuroinflammation, lipid, redox), similar to those reported in ASD [57]. Further, MacFabe has presented a developmental model of systemic PPA exposure (subcutaneous, intraperitoneal) where prenatal and postnatal exposure to PPA results in enduring ASD-like behaviors in a sexually dimorphic manner [59-61]. Furthermore *in vitro* work using rat pheochromocytoma (PC12) cells, have shown that PPA, and to a lesser extent butyrate, is capable of altering expression of a broad number of genes and genetic pathways implicated in ASD [51]. Although preliminary, these findings comprise a compelling microbiome-gut-brain connection that may, in part, be related to aberrant enteric microbiome species, their metabolic end-products or altered metabolism associated with ASD [8, 19, 32, 34, 62-65]. This naturally leads to the speculative notion of whether preservation or manipulation of the enteric microbiome or its metabolic end-products could be a meaningful and worthwhile endeavor to reduce risk of ASD or improve ASD symptoms [9]. To this end, it has also been speculated as to whether abnormal enteric gut species could play a role in the etiology of ASD, particularly regressive ASD. It may also offer a potential explanation of why certain diet manipulations may provide therapeutic benefit for certain children with ASD as modifying the gut ecosystem through dietary changes may influence the taxa or metabolic fermentation products of the microbiome.

Further, the PPA theory of ASD puts forth the compelling notion that autistic regression may be a result of multiple interacting factors that present at critical times in development. MacFabe and others have proposed that factors such as C-section, antibiotic use and hospitalization in early life, may be linked to increased risk of developing ASD through early disruption of the normal development of the infant microbiome. Of note, antibiotics can disrupt the microbiome as well as the gut and metabolic systems in several

ways to promote disease. Recurrent antibiotic use is well known to increase the colonization of clostridia species, particularly *Clostridium Difficile*. However, long term administration of antibiotics (e.g. beta-lactams) for routine pediatric infections (i.e., upper respiratory infections) can also disrupt carnitine metabolism and directly inhibit mitochondrial function and carnitine uptake, resulting in detrimental metabolic effects [19, 66]. Furthermore, antibiotics can have proinflammatory effects in the gut by impairing the activity of tight junctions and increasing the diffusion of immunogenic molecules from the gut lumen into the body. All of these factors could combine to cause immune and metabolic disturbances especially if certain other genetic and environmental factors exist. Fig. 3 outlines the broad physiological effects of PPA.

If the PPA model holds to be valid, then there is the theoretical potential that at least some forms of ASD may comprise a family of inherited and/or acquired metabolic disorders that are potentially treatable and possibly even preventable. In later sections of this review, we will touch on treatments that target certain aspects of the PPA model and their efficacy at addressing certain metabolic and behavioral features of ASD.

Cell Danger Response (CDR) Theory

The Cell Danger Response Theory (CDR) theory put forth by Naviaux [12, 37, 67] refers to an adaptive response by the cell to a perceived threat. The potential pathological nature of this response occurs when the CDR becomes chronic (i.e. persisting after the threat has dissipated). The CDR presumes that extracellular adenosine triphosphate (ATP) is a cellular purinergic signal for external "danger". Since, under normal conditions, ATP is predominantly intracellular, the presence of this molecule in the extracellular environment is an indicator of local cellular (tissue) destruction. Thus, when non-damaged cells detect extracellular ATP they shift cellular functions toward a protective cellular response repertoire. These changes include changes in cellular electron flow, oxygen consumption, redox metabolism, membrane fluidity, lipid dynamics, bioenergetics, carbon and sulfur resource allocation, protein folding and aggravation, vitamin availability, metal homeostasis, pterin, and 1-carbon metabolism, and polymer formation [12]. If the CDR remains activated, then systemic metabolism may be disrupted,

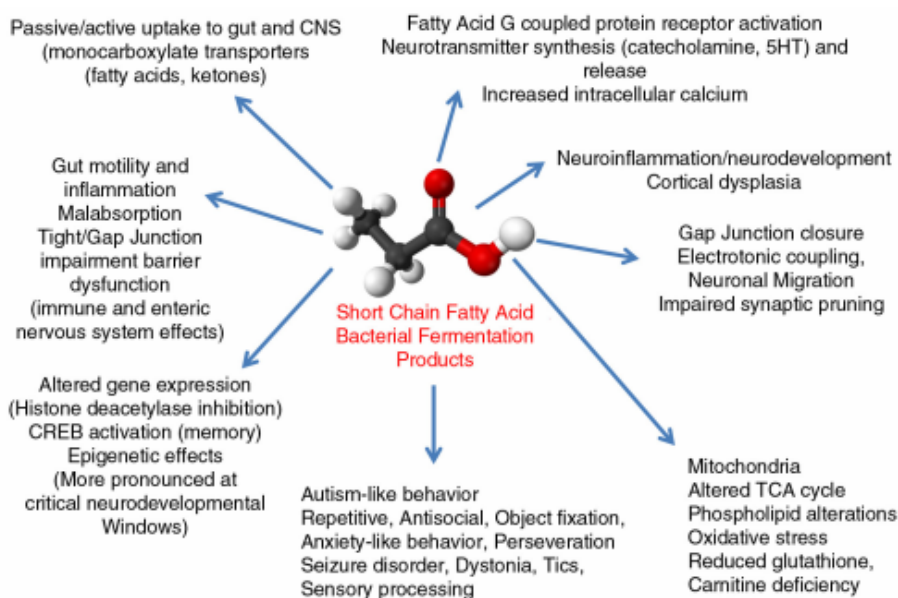


Fig. (3). The broad physiological effects of Propionic Acid (PPA). From MacFabe [51] with permission.

resulting in alterations in the enteric microbiome and the initiation of chronic disease processes.

The proposed triggers for the CDR in contemporary society include changes in human migratory patterns, diet, toxin exposures and cultural activity that have occurred over the past 150-300 years. The CDR proposes an imbalance between cellular physiology and the modern environment, a so-called “metabolic mismatch”. In an evolutionary sense, the CDR posits that the surrounding ecosystems have changed faster than species can adapt. Thus, the CDR predicts that cells are adapting to external changes by alterations in physiology such as upregulation of mitochondrial function and altering epigenetic regulation [68, 69]. If this theory holds true, it could account for a unique pattern of mitochondrial dysfunction that has been associated with ASD by multiple groups [7, 57, 70-81].

To test the proposed connection between the CDR and ASD, Naviaux et al used two well-accepted mouse models of ASD, one environmentally-triggered model of ASD, the poly(I:C) mouse model [37], and a genetic mouse model of ASD, the FMR1 knockout (KO) model. The FMR1 KO is a model of Fragile X syndrome, a genetic disorder with a high rate of ASD [82]. ASD behaviors were reduced in both mouse models of ASD using the antipurinergic, antiparasitic and antifungal drug suramin. Suramin is thought to antagonize extracellular ATP signaling, thereby reducing the key signal which initiates and maintains the CDR [83]. In the Fragile X mouse, suramin not only corrected behavior but also corrected the pathophysiology associated with ASD, leading to improvements in metabolism (e.g. purines, microbiome, *s*-adenosylmethionine/*s*-adenosylhomocysteine (SAM/SAH), glutathione, nicotinamide adenine dinucleotide (NAD⁺), fatty acid oxidation, glycolysis, eicosanoids, gangliosides, cholesterol metabolism, phospholipids) and synaptic structural abnormalities [82]. Both behavioral and metabolic abnormalities were also corrected in the poly(I:C) mouse model. Using these two models Naviaux et al. showed that both environmental and genetic factors that cause ASD-like symptoms in animals have metabolic components consistent with the CDR theory, and that these metabolic abnormalities can be remediated by antagonizing extracellular purinergic signaling (i.e. extracellular ATP). Further, both models showed that one of the primary metabolic shifts related to suramin treatment, which presumably leads to behavioral changes, was through alteration of metabolites from the microbiome. As Naviaux predicts that the CDR shifts full body

metabolism and in turn alters the host microbiome. The evidence for suramin altering extracellular purinergic signaling and host microbiome is promising.

It is significant to note that a single dose of suramin also resolved autistic-like behaviors in an adult mouse model of ASD, which may mean that, at least in rodents, if the underlying core biological process can be addressed, then timing related to when the intervention is given may not matter [82]; this challenges the traditional concept in medicine of a “static encephalopathy” which arises from that idea that neurological circuits are irreversibly “hard wired” if critical time windows of development are passed without achieving certain developmental milestones. These are promising findings that need to be confirmed in humans. To this end, a small clinical trial has examined the safety of suramin in patients with ASD (clinicaltrials.gov/ct2/show/NCT02508259). The results of this trial will be highly anticipated.

POTENTIAL TRIGGERS FOR ASD AND POTENTIAL THERAPEUTIC INTERVENTIONS

As the above theoretical models for ASD have shown, ASD may be viewed as a “whole body” or systemic metabolic disorder that may be triggered at certain points during development when there is excessive stress on the developing organism’s physiological systems. Disruption in multiple physiological systems may converge to result in the development of an ASD phenotype. Physiological systems that are believed to be involved in this process include redox metabolism, mitochondrial dysfunction, the immune system, epigenetic processes and the enteric microbiome. Disruption in any one system may affect the other interconnected systems due to their feedback mechanisms and crosstalk, leading toward an altered neurodevelopmental trajectory.

In this section, we will review certain triggers that have been proposed which may be a “hit” on the developing organism that may then, in turn, alter these interconnected physiological systems and increase risk for an ASD. No one factor, except in rare events, may be enough to trigger an ASD. Indeed, genetic susceptibility may interact with the timing and frequency of potential triggers. Triggers are believed to work in concert and be additive or multiplicative, such that each additional trigger increases risk by a certain amount to increase vulnerability to the physiological systems

by adding stressors on them which in turn may increase the risk of developing ASD.

Mitochondrial Inhibitors (Toxins and Medications)

Many classes of drugs effect mitochondrial function and may be toxic to the mitochondria. These include certain anesthetic agents, antipsychotic medications, certain classes of antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), anticonvulsants, statins, steroids, proton pump inhibitors (i.e., Omeprazole) and certain cancer medications [66, 84, 85]. Other known mitochondrial toxins include pesticides and herbicides that have been theorized to play a part in ASD etiology and/or pathophysiology [86-92]. While this review will not cover the full mechanisms of action and/or a detailed breakdown of each drug, drug class, and their potential effects, we will briefly acknowledge that any of these agents, if given during pregnancy and/or early postnatal life, may contribute to the risk of developing ASD.

Both classic and novel types of mitochondrial dysfunction appear to be prominent in ASD. First, there is a high rate of the diagnosis of classic mitochondrial disease in ASD compared to the general population, with a higher rate of biomarkers of mitochondrial disease [93] (See www.nature.com/mp/journal/v17/n3/full/mp2010136a.html). Secondly, studies have reported high rates of electron transport chain dysfunction in children with ASD in immune cells [94]. Third, there are several novel forms of mitochondrial dysfunction associated with ASD that may be acquired as a consequence of environmental influences. Indeed, one type of mitochondrial dysfunction is characterized by unique abnormalities in citric acid cycle, redox and acyl-carnitine profiles consistent with the abnormalities found in the PPA model of ASD [19, 57] (See www.nature.com/tp/journal/v3/n1/full/tp2012143a.html and www.microbecolhealthdis.net/index.php/mehd/article/view/27458). In another type of novel mitochondrial dysfunction, the mitochondrial respiratory rate associated with ATP production is about twice that of controls [72, 74]. Interestingly, these overactive mitochondria are sensitive to increased reactive oxygen species and it is believed this increase in mitochondrial respiration is an adaptive and/or compensatory change of the cell to protect itself from potentially harmful environmental triggers [70, 71, 74] (See www.nature.com/tp/journal/v4/n4/full/tp201415a.html and [www.journals.plosone.org/plosone/article?id=10.1371/journal.pone.0085436](http://www.journals.plos.org/plosone/article?id=10.1371/journal.pone.0085436)).

Whether mitochondrial dysfunction is an etiological risk factor for ASD or whether it is a pathophysiological process that results from the underlying process that causes ASD is unknown, but it is interesting in that the CDR predicts that the mitochondria would be in a hyperactive state. The fact that mitochondrial dysfunction appears to only affect a subset of children with ASD and not the whole ASD population highlights the fact that many subsets of pathophysiological processes that are most likely involved in ASD. Indeed, this may be a reason why the CDR may only be partially correct and other theories, such as the BDT, 4-EPS and/or PPA theory, may be complementary. Furthermore, these theories may not be mutually exclusive theories and may overlap.

It has been suggested that mitochondrial toxins and/or modifiers may be potential risk factors for ASD (See Table 1). Interestingly, the only two FDA approved medications for ASD are solely indicated for non-core behavioral abnormalities (i.e. irritability) associated with ASD. Both of these new-generation “atypical” antipsychotic medications have detrimental effects on mitochondrial function [95-97]. The adverse effect profiles of atypical antipsychotics include weight gain, alterations in lipid and glucose metabolism and increases the risk for obesity, metabolic syndrome, tardive dyskinesias and type II diabetes [98-102]. These prominent adverse effects no doubt involve deleterious effects on mitochondrial function, although limited research has examined the role that the mitochondria play in adverse effects of antipsychotic medications. Thus, it is

Table 1. Known Iatrogenic Mitochondrial Toxins/Inhibitors [84].

Protophonic Uncouplers	Diclofenac, Diflunisal, Flufenamic Acid, Ibuprofen, Indomethacin, Naproxen, Nimmesulfide, Pirprofen, Tetracyclines, and Tolefenamic Acid
Electrophoretic Uncouplers	Amineptine, Amiodarone, Aspirin, Bupicacaine, Buprenorphine, Chloropromazine, Chloroquine, Dibucaine, Fluoxetine, Imipramine, Perhexiline, Pentamidine, Propofol, Propranolol, Quinidine, Tarcine, Tamoxifen, Tianeptine, and Tolcapone
Redox Cycle Uncouplers	Doxorubicin, Gentamycin, Fluoroquinolone, Isoniazid, and Menadione
Complex I Inhibitors	Amytal, Haloperidol, Chloropromazine, Fluphenazine, Risperidone, Clozapine, Nefazodone, Clofibrate, Fenofibrate, Ciprofibrate, Troglitazone, Rosiglitazone, Pioglitazone, Metformin, Phenoformin, Buformin, Bupivacaine, Lidocaine, Halothane, Flutamide, Dantrolene, Phenytoin
Complex II Inhibitors	Cyclophosphamide, Ketoconazole, Hydrazine, Isoniazid
Complex III Inhibitors	Acetaminophen, Isoflurane, Propofol, Sevoflurane
Complex IV Inhibitors	Cephaloridine, Cefazolin, Cefalotin, Tamoxifen
Complex V Inhibitors	Oligomycin, Propofol

of supreme importance to find biomarkers that may predict which children are more likely to be affected negatively by these medications. Interestingly, a recent study showed that the weight gain resulting from olanzapine, an atypical antipsychotic, was associated with microbiota disturbances and that weight gain was attenuated by antibiotic administration in a rat model [103]. Furthermore Bahr *et al* [104] showed that risperidone, another second generation atypical antipsychotic, demonstrated increased weight gain and an altered enteric microbiota in children via decreased ratios of Bacteroidetes:Firmicutes when compared to antipsychotic naive children. Interestingly, this ratio has also been correlated to the development of obesity and risk for Type II diabetes and the inverse ratio has been associated with weight loss [105, 106].

Bactericidal antibiotics (quinolones, aminoglycosides, and β -lactams) induce reactive oxygen species (ROS) and cause mitochondrial dysfunction in mammalian cells. This effect can be blunted with pre-treatment with N-acetyl-L-cysteine (NAC) or through the usage of bacteriostatic antibiotics [107]. Interestingly, Rose *et al.* [70, 71] demonstrated that ROS induced mitochondrial dysfunction in lymphoblastoid cell lines (LCLs) from children with ASD could be prevented with NAC pretreatment. It is possible that ROS produced by bactericidal antibiotics during illness may induce mitochondrial dysfunction and that NAC could be given in combination with the antibiotics to minimize the negative effects on host mitochondria and possibly even prevent antibiotic induced metabolic disturbances.

In addition, the use of both beta lactam antibiotics and omeprazole [66], which are commonly used for routine infections and gastroesophageal reflux, respectively, impair carnitine metabolism and deserve re-evaluation as potential contributors to mitochondrial dysfunction and ASD, particularly the regressive-subtype.

Acetaminophen

Acetaminophen is widely used in obstetrics and pediatric medicine. Although it is an approved medication in North America for over a century, its mechanism(s) of action are still not fully elucidated. Recently, its safety and efficacy has come into question. Indeed, its effectiveness as an anti-inflammatory and/or antipyretic has demonstrated questionable efficacy and effectiveness to date and has been debated for some time. It is important to note that other agents have demonstrated similar and/or better safety and tolerability profiles for chronic use [108]. Acetaminophen has also recently been under scrutiny related to recent associations between prenatal exposure and hyperkinetic and attention deficit hyperactivity disorders [109, 110]. As emerging evidence is starting to show through multiple empirical pre-clinical studies, case reviews, and theoretical models, acetaminophen may have potentially catastrophic consequences on the development of metabolic and neural systems if used during pregnancy or during the perinatal or postnatal period. If acetaminophen's risk profile has changed over time or whether we are better at detecting problems now is of considerable debate, but if biome depletion and missing microbes are to play, then there should be evidence that shows its effects on the microbiome and its interconnected metabolic constituents. Interestingly enough, p-Cresol, the human analogue to 4EPS described by Hsiao et. al [25], which has been shown to be elevated in urine of patients with ASD has been linked to acetaminophen toxicity via individual variations in the microbiome [111]. *Clostridium difficile* metabolizes tyrosine to p-cresol which competes with acetaminophen for sulfuration in the gut. Researchers found that the ratio of sulfonated to glucuronated p-cresol in the urine was predictive of acetaminophen toxicity [112, 113].

Because of the poor glutathione and methylation status in children with ASD and their first-degree relatives [114-117], individuals with ASD and their relatives may be especially vulnerable to acetaminophen's effects on sulfuration as described above.

It is interesting to note that the etiological basis of ASD has been speculated to occur via activation of the endocannabinoid system (ES) by acetaminophen at critical time points during development [118]. Another interesting finding is that some children with ASD seem to have transient improvements in their ASD symptoms during bouts of fever [119-122]. Since activation of the CB₁ receptors appear to be involved in the fever response, it is possible that the ES may be activated in ASD during fever [123, 124].

Furthermore, the PPA rodent model has also shown impairments in glutathione redox metabolism suggesting that SCFAs may render an individual more sensitive to further glutathione depletion via acetaminophen exposure, and result in further sensitivity to a broad range of xenobiotics [19, 36, 51].

As acetaminophen may be poorly metabolized by a subset of children with ASD [125] and there may be physiological disruptions that have been associated with ASD that would make individuals with ASD poor metabolizers in the first place, it may be wise to limit its use in individuals with ASD.

Furthermore, due to the many possible mechanisms by which acetaminophen may promote the formation of ASD, we would strongly suggest that it be critically re-examined for its use in obstetric and pediatric care due to its potential effects on the developing fetus and child.

Maternal and Child Folate Metabolism

Many lines of evidence have pointed to the importance of pre-conception and prenatal folic acid in the prevention of ASD [103,

126], however there are inconsistent results [127] which may have arisen due to many factors (e.g. abnormalities in genetic, epigenetic, and/or immune system function, etc.) [128]. Folate status is complex and is affected by diet [129] and autoantibodies that may affect its transportation into the brain and across the placenta [130, 131]. Genetic polymorphisms in folate metabolism in children and their mothers modulate the risk of developing ASD [132]. Furthermore, folate is produced by the microbiome. If there are disruptions in the mother's microbiome during pregnancy, this may impact folate production and in turn lead to increased risk for ASD and explain why higher doses of folates protect against developing ASD. Thus, the number of factors that may influence folate metabolism to increased ASD risk is complex.

It is important to understand that Folic Acid is an oxidized form of folate that needs to be reduced in order to be utilized by the body. Frye et al [133] found a high percentage of folate receptor alpha autoantibodies in children with ASD. Many of the mothers of the children also demonstrated these autoantibodies. Interestingly, this autoantibody blocks the transport of folate into the brain, requiring treatment with a reduced form of folate known as folinic acid (leucovorin calcium). Children with ASD treated with folinic acid in an open-label fashion for 3 months at 2mg/kg/day in divided doses demonstrated a significant positive behavioral responses in core ASD symptoms [133]. This data has been further validated in both a double-blind placebo controlled and open-label clinical trials [134, 135]. These data suggest that reduced folates may be advantageous and therapeutic to children with ASD since they may restore perturbations in folate metabolism. Likewise, since many of these same abnormalities have been found in mothers of children with ASD, reduced forms of folate may be advantageous for some women to decrease the risks of having offspring with ASD. In any case, further study on folate derivatives, which may be able to treat abnormalities in folate metabolism in children and mothers are warranted.

One area of research that is ripe is the empirical examination of folate pathway abnormalities, including folate receptor alpha autoantibodies and/or polymorphisms in folate and folate-related genes in both mother and offspring in order to formulate treatment plans which minimize the effect of dietary and other environmentally triggering factors (e.g. acetaminophen usage [109, 110, 118, 136-139], types of prescribed antibiotics [107], avoidance of pro-inflammatory foods [140-143], bovine milk [129], etc.). One important strategy would be to prophylactically treat the child and/or mother with a reduced type of folate, such as folinic acid, either to prevent ASD all together or decrease symptom burden and improve outcomes. Further, as mentioned earlier, since the mother's microbiome may be disrupted and alter folate production, this may be another reason why high dose folates may be advantageous. While these points are purely theoretical at this time, it would not be a difficult study from a methodological stance to follow large cohorts longitudinally to see if ASD incidence and prevalence rates go down in response to the proposed strategies listed above.

Gestational Factors That Influence ASD Risk

While folate has been documented to have a significant role during pregnancy in contributing to ASD risk, there are other factors that have been suggested in the literature that are also important. These will be reviewed below.

Maternal Infection

Several well-validated rodent models of environmentally acquired ASD involve maternal infection or inflammation during pregnancy [144, 145]. These models demonstrate sexual dimorphism consistent with the higher male prevalence of ASDs. The exact mechanisms for how maternal immune activation during pregnancy can give rise to a heterogeneous condition such as ASD is of much debate, but many lines of evidence support the idea that maternal immune activation may give rise to alterations in synaptic

plasticity via alterations in glial cell function (i.e. astrocytes and microglial cells) through immune mediated means and promote neuroinflammation and neuronal dysfunction [146]. These factors may alter neuronal migration, cortical pruning and the excitatory/inhibitory balance of the developing brain, all of which have been suggested in ASD [147].

Maternal immune activation can alter the expression of important genes through epigenetic modification. For example, using the poly(I:C) mouse, Labouesse et al demonstrated that a prenatal immune challenge altered expression of the promoter regions of GAD1 and GAD2 genes. This resulted in decreased expression of genes that code for the GABA-synthesizing enzyme glutamic acid decarboxylase, an enzyme essential for inhibitory GABA neurotransmission in the brain. Such changes were accompanied by impairments in working memory and social interaction [148].

One study has suggested that there is a clear genetic predisposition to those children who are susceptible to maternal infection. Mazina et al demonstrated that children with ASD that had both a history of chromosomal copy number variations and gestational infection demonstrated worse social communicative and behavioral impairments as compared to others with ASD [149].

Maternal infection may also increase risk for disrupting the vaginal and/or placental microbiomes and alter the fetal microbiome [150-154]. It has been shown that disruption of the vaginal microbiome via decreased *Lactobacillus* species may increase risk for preterm birth and low birth weight, both of which have been associated with increased risk for ASD and other neurodevelopmental conditions [155, 156]. Furthermore prenatal and early postnatal exposure to lipopolysaccharide, an activator of the innate immune system, potentiates the behavioral effects of the enteric bacterial metabolite PPA in rodents [59, 60].

As mentioned, during pregnancy it is not uncommon to treat infections with antibiotics or use acetaminophen for pain or fever, or omeprazole for gastroesophageal reflux. These, and other, iatrogenic factors could potentially alter the neurodevelopmental trajectory of the developing fetus if it occurs at critical and vulnerable points during gestation and may alter metabolism and affect the microbiome.

Maternal Autoimmune Reaction

One of the more interesting findings in ASD research over the past 10 years is maternal autoantibody-related (MAR) autism as described by Van de Water et al [157-159]. MAR involves maternal autoantibodies that target fetal brain proteins and are specifically associated with the development of autistic disorder. These autoantibodies are believed to cross the placenta and impact brain development during pregnancy and have been estimated to be present in a significant subset of mothers of children with autism. Interestingly, injecting these antibodies into non-human primates during the 1st and 2nd trimester of pregnancy results in detrimental effects on the monkey's brain development and social behavior [159]. Indeed, monkeys display ASD like behaviors after the injection of the antibodies to their mothers during pregnancy, providing some compelling evidence these autoantibodies may play a significant role in the development of ASD. However, questions remain regarding the origins of the autoantibodies or what can be done therapeutically to minimize or eliminate their effects.

It could be possible that an abnormal maternal enteric microbiome could contribute to an autoimmune reaction and alter the gut-brain axis through an altered intestinal lining or a permissive entrance of immunogenic molecules secondary to impaired tight junctions [160-163], but this as yet has not been tested. If this holds to be true then it raises the possibility that straightforward treatments such as customized prebiotic and probiotic blends and/or fecal microbiota transplantation (FMT) could reduce autoimmunity. In this manner, modulation of the enteric microbiome and its metabolic end products could potentially offset the antibodies deleterious

effects via altering immune system reactivity. This would require rigorous investigation with replication in clinical trials and long term clinical follow-up, even if preliminary evidence supports the notion.

Psychological Stress Management

Alterations in the gut microbiome of germ-free mice have been shown to exaggerate the stress response by activating the hypothalamic pituitary axis (HPA). Colonization with microbes such as *Bifidobacterium infantis* or *Lactobacillus* [164-167] can normalize this response. Thus, it is plausible that chronic stress may be caused by disruptions in the enteric microbiome [168].

Furthermore, a recent study showed that mitochondrial function modulates neuroendocrine, metabolic, inflammatory, and transcriptional responses to acute psychological stress in a mouse model [169]. This is predicted by the CDR theory. Interestingly, mitochondria can become dysfunctional when presented with oxidative stress, and impact methylation and glutathione metabolism, leaving the mitochondria in a vulnerable state. This could theoretically tie back in via bidirectional feedback loops and promote a negative synergy, influence the microbiome and its metabolites, and promote pro-inflammatory cascades. This may all be linked to the mitochondria being the driving force behind the disrupted developmental trajectory and psychological stress being a key driver of this dysfunction. In addition, the emerging role of PPA and related SCFAs on specific G-coupled receptors on Treg cells in the gut and autonomic nervous system provides further potential mechanisms where the enteric microbiome can alter host immunity [170].

The disruptions of multiple interconnected physiological systems could lead to a loss of *complexity* and potentially alter development. Interestingly the oxidative and mitochondrial theories of aging have been hypothesized in senescence and aging [171]. In fact a theory of ASD being a form of accelerated development which could be analogous to breakdowns in physiological systems associated with aging has been described [172].

It has yet to be seen whether prenatal prevention strategies that incorporate interventions such as probiotics, meditation, and yoga can significantly alter the microbiome, host immunity and stress response and decrease risk for ASD, but since chronic psychological stress may have a direct effect on the developing fetus, these interventions theoretically speaking, could act synergistically to improve pregnancy and post pregnancy related outcomes. A recent systematic review of randomized controlled trials (RCTs) related to prenatal yoga [173] found that yoga may reduce pelvic pain and may possibly improve mental and physical conditions as well as perinatal outcomes. Although there have been studies that show that meditation can alter inflammation, gene expression, and enhance neuroplasticity [174-179], studies on the effect of mediation on the microbiome and ASD risk in the offspring has not been evaluated.

As Picard et al [169] have demonstrated, the multisystemic response to acute psychological stress via a restraint mouse model was mediated by bioenergetic and redox metabolism. Furthermore, mitochondrial dysfunction alters and modulates the stress response so that if the mitochondria are dysfunctional the response to psychological stress may be exacerbated.

Furthermore, it has been shown that the relaxation response, which is the inverse of the stress response, induces time dependent transcriptome changes in mitochondrial and inflammatory pathways, as well as insulin secretion [180]. This implies that increased relaxation via meditation, yoga, and/or repetitive prayer could theoretically aid in pregnancy related stress responses. Alternatively, it could also be that psychological and/or physical stress during pregnancy could alter mitochondrial function in the mother and promote abnormalities in these mitochondrial related pathways which could influence the development of the fetus and theoretically could increase the risk for ASD.

Dietary Contributions

Proinflammatory (high fat) diets during pregnancy could theoretically alter the enteric microbiome and influence fetal brain development via altering microglial activity through proinflammatory processes or neuroendocrine disruption [142, 143]. A seminal study in microbiome research published in 2010 by De Filippo et. al [181] demonstrated that children of the rural African village of Burkina Faso had significantly more diverse microbial compositions than children of European descent. The authors concluded that the differences seen in the microbiomes were related to post-weaning dietary differences and that the Burkina Faso cohort had more diverse and “healthy” microbiomes than their European counterparts. This finding provided evidence that a Westernized Diet (i.e. high fat low fiber) may contribute to a less diverse and less “healthy” microbiome and that dietary contributions are paramount to the development and maintenance of a healthy microbiome.

Sonnenburg et. al. recommends diets rich in microbiota accessible carbohydrates (MACs) as a “microbiome friendly” diet and one that is far superior to the Westernized diet which may increase inflammatory disorders by altering the microbiome [182]. MAC diets alter SCFAs (particularly butyrate, as opposed to propionate) profiles by increasing inulin containing foods and reducing refined carbohydrates. MAC diets also attenuate inflammation by increasing complex carbohydrates rich in phytonutrients and reducing refined carbohydrates. Another recent study by Zeevi et al. demonstrated that individualized differences in response to food consumption might be influenced by the microbiome [183]. In other words, a diet that may be good for one person may be bad for another depending on their genetics/epigenetics and microbiome composition. Whether or not this approach can be implemented before or during pregnancy to promote a healthy microbiome during pregnancy and in improve outcomes of the offspring is currently unknown.

A recent animal study showed that Westernized diets before or during pregnancy can lead to transgenerational extinction events in which the offspring could not acquire certain microbial populations after birth presumably due to altered immune responses [184]. Furthermore, extinction events related to low MAC diets may be recovered in a single generation but if multiple generations of extinctions occur, re-introduction of high MAC diets may not be sufficient in and of itself to restore a healthy microbiome; in such cases it will be necessary to administer missing taxa in combination with a diet that is high in dietary MACs [185]. Therefore, it may be necessary to include other therapeutic strategies such as FMT, or defined human gut microbial communities [22], in combination with high MAC diets to restore a disrupted microbiome. This is consistent with other lines of research that refer to missing “keystone” species and the “missing microbiota hypothesis” that theorize that modern lifestyles result in the development of an unhealthy microbiome with less diversity [186].

Since dietary changes can be relatively easy to implement and could potentially modify disease risk, this may be an area worth exploring in large randomized controlled trials. Thus, developing biomarkers to identify missing taxa may have merit and be beneficial. Missing taxa could be modified by FMT, but careful selection of donors is extremely important to ensure unintended effects, such as transmission of antibiotic resistant bacteria and viruses or other untoward latent effects such as increased weight gain [9, 187, 188].

Vitamin D Deficiency and Tryptophan Metabolism

Many scientific inquiries and studies have questioned whether vitamin D deficiency is a contributing factor to ASD [189-192]. Since vitamin D, a secosteroid, upregulates DNA-repair genes, deficiency in vitamin D may lead to inability to repair *de novo* mutations [193]. Since vitamin D [192] activates the transcription of the serotonin-synthesizing gene tryptophan hydroxylase 2 (TPH2) in the brain, vitamin D deficiency may explain reduced serotonin in the brains of children with ASD. Vitamin D deficiency may also

explain elevated serotonin in the periphery as it represses the transcription of TPH1 in the periphery of those affected with ASD. Patrick and Ames has suggested that this could lead to abnormal tryptophan metabolism and could also account for maternal autoantibodies as well as the high male prevalence in ASD [192, 194, 195]. Since estrogen can rescue the repression of TPH2 caused by vitamin D deficiency, girls may be protected, leading to this effect predominantly affecting boys. Poor vitamin D status also causes overexpression of TPH1 which results in an increase in tryptophan degradation. This, in turn, decreases the production of T-regulatory cells which could result in maternal autoimmunity and the overproduction of antibodies to the fetal brain.

Research has also shown effects of tryptophan metabolism and vitamin D deficiency on the host microbiome. A study by Reigstad et al demonstrated that human microbiota may upregulate TPH1, the rate limiting enzyme for the production of 5-HT, the precursor to serotonin. Additional studies suggested that this effect occurred through the production of SCFAs [196]. Further studies have shown that microbiota dysbiosis during disease processes may lead to altered tryptophan metabolism that can exacerbate or give rise to disease conditions [197]. Vitamin D deficiency has also been shown to alter the epithelial barrier, lead to intestinal inflammation, and alter the host microbiome [198, 199].

It has been theorized that supplementation with vitamin D and tryptophan may be a cheap, practical, and easy solution to try and prevent ASD and/or ameliorate certain symptoms.

Cesarean Sections (C-Sections)

It has also been suggested that C-Sections may be a risk factor for ASD, although the data to support this is not strong at present [200, 201]. Infants born by C-Section are not exposed to the vaginal flora at birth, and may be colonized by maternal skin or hospital acquired microbiota. This lack of exposure to maternal vaginal and intestinal flora could alter the development of the enteric microbiome which may, in turn, influence neurodevelopment. In fact, a recent study by Dominguez-Bello et al partially restored the microbiota of C-section born infants via vaginal microbial transfer at birth. However, the long-term outcomes and neurodevelopmental benefits of this practice are currently unknown [202]. If this proves to be beneficial, there could be practical implications for C-Section practices. Babies born via C-Sections could be colonized with vaginal microbiota at the time of delivery [202, 203]. Interestingly, infants born by C-sections have a lower likelihood of being colonized by *B. infantis* which is a prominent constituent of a healthy vaginal microbiome.

Other Potential Gestational Risk Factors

Other possible factors that could influence the maternal microbiome and affect infant development that could be putatively related to the microbiome include prenatal toxin exposure and maternal sleep hygiene. These will be reviewed below.

Prenatal Toxin Exposure

A systematic review of toxin exposures and ASD has recently been published [91]. Toxins associated with ASD include, pesticides, air pollution, solvents, polychlorinated biphenyls (PCBs), phthalates, bisphenol A, herbicide and mercury exposure as well as cigarette smoking, illicit drug use, heavy metals and alcohol exposure. All of these exposures could potentially impact redox regulation and promote oxidative stress, which have been reported in ASD parents and children [116]. The impact on redox and detoxification systems could influence mitochondrial function, promote greater oxidative stress, impact the enteric microbiome and create further metabolic and immune dysfunction, alter genetic expression via epigenetics, cause *de novo* genetic mutations as well as alter neurodevelopmental specific processes such as neural migration, neurotransmitter synthesis and release, microglial activity, myelination, and axon growth [147].

Maternal Sleep Hygiene

Disrupted circadian rhythms can affect stress responses, a common factor in neuropsychiatric disease [204, 205]. Furthermore, sleep is intimately related to metabolic function and alterations in sleep is well-known to promote cognitive dysfunction, mental health, immune function, weight, cardiovascular health, and other affiliated health concerns. Recently it has been shown that there is significant diurnal variation in the host microbiota which is associated with sleep [206-209]. Given the many ways in which sleep disruption could influence host metabolism and the microbiome, maternal sleep hygiene should be considered a potentially modifying factor.

Breastfeeding (Neonatal / Early Life risk factor)

An extremely important influence on colonization and the constitution of the enteric microbiome is breast feeding. Breast milk is a primary prebiotic source that provides a rich source of oligosaccharides and is a known major postnatal contributor to the development of the immune system [210]. While the infants are unable to digest these oligosaccharides, important bacteria such as *B. infantis* utilize these carbohydrates to help establish a diverse infant microbiome. As stated above, the first colonization with *B. infantis* comes from the vaginal canal, if the baby is vaginally delivered. Insufficient perinatal breastfeeding is correlated with subsequent development of ADHD [211]. There is both positive and negative data linking insufficient breastfeeding to ASD risk [212, 213]. As stated earlier, it's also important to note that there is probably no single factor, except for in rare events that, in isolation, accounts for ASD. Therapeutically speaking, breastfeeding is a safe practice that may contribute to the formation of a healthy microbiome that may decrease ASD risk.

Other Perinatal and Postnatal Factors

The development of the human microbiome begins before birth and at age 3 years old appears to resemble the adult microbiome [214]. It may be that the critical timeframe to establish the microbiome may be as early as the first 28 days of life. Since there seems to be a correlation between the development of the microbiome and the development of ASD, the gestational factors listed in this review should also be considered as possible environmental factors that could influence ASD risk in the perinatal and postnatal setting as well. Therefore, caution related to these factors is warranted, although evidence is still needed to firmly associate them with ASD risk.

Summary for Prenatal and Postnatal Risk Factors for Autism Spectrum Disorder

Above we highlight certain important triggering events in the prenatal, neonatal, and early life related to metabolic alterations that have been linked to ASDs through various interconnected pathways including the enteric microbiome, mitochondrial function, and oxidative stress related interactions. Table 2 below outlines possible remediation strategies that may decrease ASD prevalence by minimizing the effects on the developing child's metabolic systems.

CONCLUSION

ASD is a heterogeneous neurodevelopmental disorder of unknown origin and little is known about its etiology and effective treatments. While emerging research is starting to shed light on the multiple physiological systems that may contribute and interact with triggers leading to the formation of ASD, much more research is needed to further elucidate the disease mechanisms at play. In this manuscript we outline three primary theories related to the etiological basis of ASD and enteric microbiota and macrobiota disruptions. These models highlight the interconnected feedback loops between the mitochondria, oxidative stress, immune function,

Table 2. Remediation Plan for Decreasing Pre/Postnatal Factors that Influence ASD Risk by affecting Mitochondrial Function, Redox Metabolism, and the Enteric Microbiome and Macrobiome.

Potential Trigger	Possible Remediation Plan
Mitochondrial Toxins	Avoid and Eliminate [see 4a and 4b] If certain medications can't be avoided a mitochondrial cocktail such as carnitine, Co-Q10, B Vitamins, Creatine Monohydrate, and anti-oxidants [49]
Antibiotics (Abx)	Provide Pre/Probiotics with Abx and/or NAC. Supplement with carnitine if using β -Lactams
Acetaminophen	Avoid and Eliminate. Pre-treat with NAC if unavoidable.
Poor Folate Metabolism and/or Absorption	Treat with reduced folates (e.g. folinic acid) and avoid folic acid
Bovine Milk Products	Eliminate or Minimize Use
Maternal Infection	Minimize exposures and medications that could further complicate development
Maternal Autoimmune Reaction	Pre/Probiotic Supplementation and other immune supporting agents
Psychological Stress Management	Meditation, Yoga, and/or other relaxation techniques to mothers during pregnancy.
Pre/postnatal Toxins	Avoid air pollution, solvents, polychlorinated biphenyls (PCBs), phthalates, bisphenol A, and mercury exposure as well as cigarette smoking, illicit drug use, and alcohol exposure
Poor Diet	Eat foods high in microbiota accessible carbohydrates along with fruits and vegetables. FMTs in the future may be warranted
Premature Weaning and/or Formula feeding	Breastfeed for at least 6 months and/or supply breast milk from donors over formula feeding or pasteurized milk
Vitamin D and/or Tryptophan Metabolism Deficiency or Disorders	Supplement with Tryptophan and Vitamin D
Poor Maternal Sleep Hygiene	Introduction of Sleep Protocols to decrease sleep associated complications
C-Sections	Avoid elective C-Sections and reserve for emergency situations only
Helminths	Re-introduction of helminthes into the intestinal ecosystem is necessary to re-establish the balance of the ecosystem

and their respective consequences. We also outline possible factors that may independently or in combination increase risk and/or be causative for ASD.

The promising news is that there may be many disease modifying strategies that are at our disposal that could be implemented to potentially reduce ASD symptomatology or prevent ASD altogether by targeting the enteric microbiome. While there have been 2 studies to date that have evaluated probiotics in ASD that seemed to promote positive changes in microbiome related outcomes [215, 216], it is unknown how effective probiotic supplementation will influence ASD symptomatology. The same can be said for other therapeutic modalities aimed at modulating the enteric microbiome such as FMT's, synbiotics, prebiotics, re-introduction of helminthes, mitochondrial supplementation, antioxidants, and/or CBD compounds.

We believe that translational animal models coupled with carefully conducted randomized controlled trials are needed to determine the safety and efficacy of any intervention to determine if it is valid, but many preventative strategies related to avoidance of possible triggers may be suitable for implementation. While these strategies are somewhat theoretical in nature, carefully conducted trials with long term follow-up are warranted and we believe that they should be implemented sooner rather than later.

LIST OF ABBREVIATIONS

4EPS	=	4-ethylphenylsulfate
ASD	=	Autism Spectrum Disorder
ATP	=	Adenosine Triphosphate
BD	=	Biome Depletion
BDT	=	Biome Depletion Theory
CDR	=	Cell Danger Response
CNS	=	Central Nervous System
FMT	=	Fecal Microbiota Transplant
GI	=	Gastrointestinal
HPA	=	Hypothalamic Pituitary Axis
ICV	=	Intracerebroventricular
LCL	=	Lymphoblastoid Cell Lines
NAC	=	N-acetyl-L-cysteine
NSAID	=	Non-Steroidal Anti-Inflammatory
PCB	=	Polychlorinated biphenyls
PPA	=	Propionic Acid
RCT	=	Randomized Controlled Trial
ROS	=	Reactive Oxygen Species
SCFA	=	Short Chain Fatty Acids

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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