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Metal Dyshomeostasis as a Driver of Gut Pathology in Autism Spectrum Disorders

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ABSTRACT

Despite being classified as neurodevelopmental disorders, in recent years, there has been a growing interest in the association between autism spectrum disorders (ASDs) and gut pathology. This comprehensive and systematic review explores a potential mechanism underlying gut pathology in ASDs, including alterations in gut microbiota, intestinal permeability, immune dysregulation, and gastrointestinal (GI) symptoms. Specifically, it delves into the role of toxic and essential metals and their interplay, affecting the development and function of the GI tract. The review also discusses the potential implications of this gut pathology in the development and management of ASDs. Studies have shown that heavy metal exposure, whether through environmental sources or dietary intake, can disrupt the delicate balance of trace elements in the gut. This disruption can adversely affect zinc homeostasis, potentially exacerbating gut pathology in individuals with ASDs. The impaired zinc absorption resulting from heavy metal exposure may contribute to the immune dysregulation, oxidative stress, and inflammation observed in the gut of individuals with ASDs. By shedding light on the multifaceted nature of gut pathology, including the impact of metal dyshomeostasis as a non-genetic factor in ASD, this review underscores the significance of the gut-brain axis in the etiology and management of ASDs.

1 | Introduction

1.1 | Autism Spectrum Disorders and Gastrointestinal Problems

Autism Spectrum Disorders (ASD) represent a multifaceted cluster of neurodevelopmental conditions characterized by difficulties in social interaction, communication, and behavior. This umbrella term includes Autism, Asperger's Disorder, and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS) (Caroline et al. 2011). ASD manifests uniquely in each individual, with a diverse array of symptoms and varying degrees of impairment. While typically identified in early childhood, diagnoses can occur at any stage of life due to the heterogeneous nature of its symptoms, characteristics, and onset (Lord et al. 2018).

Although the precise etiology of ASD remains elusive, a growing body of evidence suggests that environmental and genetic factors, including metal dyshomeostasis, play a role in its

Abbreviations: AO, Albino Oxford; ASD, Autism Spectrum Disorder; CdTe, Cadmium Telluride; CI, confidence interval; CNS, Central Nervous System; Cntnap2, Contactin-associated protein 2; DA, Dark Agouti; DMSA, dimercaptosuccinic acid; DNA, Desoxyribonucleic Acid; EDTA, ethylenediaminetetraacetic acid; ENS, enteric nervous system; Fmr1, Fragile X Messenger Ribonucleoprotein 1; GERD, gastroesophageal reflux disease; GI, gastrointestinal; IBD, inflammatory bowel diseases; IL-6, interleukin-6; LPS, lipopolysaccharides; MIA, Maternal immune activation; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; SD, Sprague Dawley; Shank3, SH3 and multiple ankyrin repeat domains 3; TNF-α, tumor necrosis factor-alpha; VPA, valproic acid; ZA, zinc adequate; ZD, zinc deficient; ZO-1, Zonulin-1; ZS, zinc supplemented.

pathophysiology (Baj et al. 2021). Most likely, ASD arises from a complex interplay of genetic, nutritional, and environmental factors. Genetic mutations affecting neuronal pathways and synaptic functions, such as those in SHANK3, NLGN3, and NRXN1, are strongly associated with ASD, with heritability in monozygotic twins estimated at 83% (95% CI: 0.79–0.87) (Daghsni et al. 2018; Sandin et al. 2017; Tick et al. 2016; Zoghbi and Bear 2012). Nutritional deficiencies, particularly in zinc (Zn^{2+}), during pregnancy also play a critical role. Zinc is essential for synapse formation and brain development, and its deficiency has been linked to impaired neuronal growth, abnormal behavior, and a higher risk of ASD (Akdas and Yazihan 2020; Bragg et al. 2022; Cortés-Albornoz et al. 2021; Gogou and Kolios 2020; Grzeszczak et al. 2020; Iqbal and Ali 2021; Vecchione et al. 2022). However, at the cellular level, genetic and environmental risk factors may converge, both, for example, impacting SHANK/NLGN/NRXN signaling at synapses.

Over the past few decades, the prevalence of ASD has risen significantly, with numerous studies pointing to geographic, methodological, and diagnostic variations as key factors influencing prevalence rates. A systematic review by Zeidan et al. identified a global median prevalence of 100 per 10,000 children (1 in 100). This estimate was based on 99 prevalence estimates from 71 studies conducted across 34 countries between 2012 and 2021. Notably, prevalence rates varied widely, ranging from 1.09 to 436 per 10,000, reflecting differences in study methodologies, diagnostic criteria, and regional influences (Zeidan et al. 2022). Similarly, Salari et al. conducted a comprehensive meta-analysis in 2022, incorporating 74 studies with 30,212,757 participants. Their analysis revealed a global ASD prevalence of 0.6% (95% confidence interval: 0.4%–1%). Subgroup analyses further highlighted regional variations: Asia (0.4%, 95% CI: 0.1–1), America (1%, 95% CI: 0.8–1.1), Europe (0.5%, 95% CI: 0.2–1), Africa (1%, 95% CI: 0.3–3.1), and Australia (1.7%, 95% CI: 0.5–6.1) (Salari et al. 2022). A three-level meta-analysis by Talantseva et al. 2023, further underscores the growing interest and need for understanding ASD prevalence trends. The increasing but varying incidence of ASD highlights the importance of identifying contributing factors and addressing disparities in diagnosis and care worldwide (Talantseva et al. 2023).

Environmental factors, including advanced parental age, maternal infections, and prenatal toxin exposure, contribute to ASD risk (Bölte et al. 2019; Lyall et al. 2017; Ratajczak 2011). The intricate interplay of genetic, nutritional, and environmental factors contributing to ASD has broadened the scope of research into potential additional influences on ASD risk and varying incidence rates.

In recent years, attention has been focused on investigating potential connections between ASD and gastrointestinal (GI) pathology, sparking the need for more research into this emerging field. A growing body of literature suggests a plausible correlation between disruptions in gut microbiota composition and GI physiology and the onset or exacerbation of ASD symptoms (Góralczyk-Bińkowska et al. 2022) (Mangiola et al. 2016) (Fattorusso et al. 2019). Individuals with ASD frequently experience a variety of GI symptoms. Abdominal pain is one of the most commonly reported GI symptoms in this population and is often described as cramping, discomfort, or generalized pain in the abdominal region (Wang et al. 2011). Constipation is another widely observed issue in individuals with ASD, characterized by infrequent bowel movements and difficulty passing stool, which can result in abdominal discomfort, bloating, and distension (Coury et al. 2012). Diarrhea, characterized by loose or watery stools, is also prevalent among individuals with ASD and may be accompanied by urgency, frequency, and fecal incontinence (Kang et al. 2014).

Besides, gastroesophageal reflux disease (GERD) is frequently observed in individuals with ASDs due to impaired esophageal function or dietary factors. Symptoms of GERD include heartburn, regurgitation, and discomfort in the chest or throat (Coury et al. 2012). Additionally, many individuals with ASD exhibit food sensitivities or allergies, which can trigger GI symptoms such as abdominal pain, bloating, diarrhea, or constipation. Common culprits include gluten, dairy, and specific additives (Adams et al. 2011).

Inflammation in the gastrointestinal tract is another resulting issue faced by individuals with ASDs. This inflammation can be characterized by mucosal damage, lymphocytic infiltration, and alterations in epithelial barrier integrity (Chaidez et al. 2014). These immune-mediated mechanisms contribute to various GI symptoms and may be associated with immune dysregulation.

Thus, individuals with ASD experience a wide range of GI symptoms, ranging from mild discomfort to severe impairment in their daily activities. These symptoms often modify behavior and communication difficulties and reduce the quality of life (Nikolov et al. 2009). Furthermore, individuals with ASDs may adopt restrictive diets or develop food aversions in response to GI symptoms or sensory sensitivities, leading to challenges in meeting their nutritional needs and exacerbating dietary deficiencies (Valenzuela-Zamora et al. 2022).

It is crucial to understand the diverse range of GI symptoms associated with ASDs and how they manifest clinically to recognize them promptly, diagnose them accurately, and manage them effectively. Exploring the intricate bidirectional communication network between the microbiota, gut, and brain, known as the microbiota –gut –brain axis, and its involvement in ASD etiology might be key to understanding risk factors for ASD and developing prevention and treatment strategies (Srikantha and Mohajeri 2019).

2 | Gut Pathologies in ASD

2.1 | Microbiome Dysbiosis

While the precise mechanistic pathways mediating the microbiota-gut-brain interplay in ASD await comprehensive elucidation, ongoing research holds promise for identifying novel therapeutic targets to modulate the gut microbiome to mitigate ASD symptoms and enhance overall patient well-being (Li et al. 2021). Emergent research posits that alterations in the gut microbiome may instigate immune dysregulation, inflammatory responses, and abnormal neurotransmitter signaling, all of which have been implicated in the pathophysiology of ASD (Kang et al. 2017). Additionally, investigations underscore the

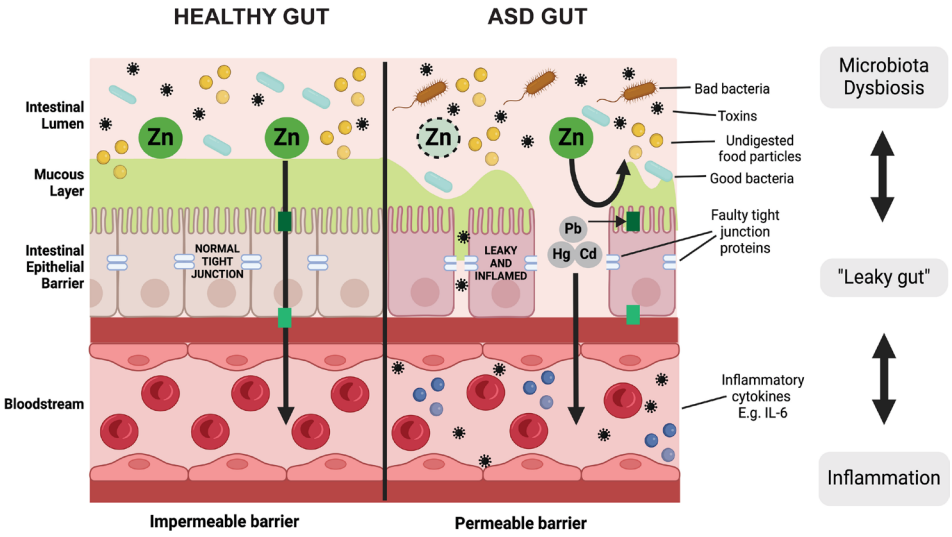

FIGURE 1 | A hypothesized mechanism linking gut health to ASD: In a healthy individual (left), the intestinal epithelium maintains a tight barrier, preventing the translocation of harmful substances into the bloodstream. Zinc is taken up through a carrier-mediated process. However, in ASD (right), compromised tight junction proteins may increase permeability, allowing bacteria, bacterial metabolites, toxins, and inflammatory cytokines to pass. Zinc is deficient or absorption is impaired (dashed line) due to the presence of and competition with toxic metals that may also leak into the blood circulation by paracellular transport. This disruption in gut homeostasis may significantly contribute to the pathology of ASD. Created in BioRender. Grabrucker (2023) BioRender.com/b00f378.

potential impact of environmental factors, such as dietary habits, antibiotic exposure, and early-life stressors, on shaping gut microbiota composition and influencing the risk of ASD onset (Parracho et al. 2005). Recently, there has been a significant increase in studies investigating the complex relationship between gut microbiota and ASD (Li et al. 2017). Emerging evidence from these studies has suggested that alterations in the signaling of the microbiota-gut-brain axis play a fundamental role in brain development and functionality (Hsiao et al. 2013).

This intricate signaling pathway involves several factors, with the microbiome emerging as a pivotal influencer in recent times. The microbiome comprises a myriad of microorganisms and exerts its impact on brain development through multifaceted mechanisms within the gut –brain axis, including modulation of the immune system, the release of microbial metabolites, regulation of tryptophan metabolism, and direct modulation of the vagus nerve and the enteric nervous system (ENS) (Cryan and Dinan 2012; Sharon et al. 2016).

The human microbiome's composition varies across distinct parts of the body, such as the gut, skin, and vagina, each subject to unique environmental factors, such as oxygen levels, moisture, pH, and microbial interactions (Belkaid and Hand 2014). These variances shape the microbial species present and their interactions with the host, consequently influencing overall gut-brain signaling.

For example, a book chapter by Sauer et al. highlights microbiota changes associated with ASD across different bacterial phyla in humans and mice. *Actinobacteria* levels show an increase in mice but vary in humans, with studies reporting both increases and decreases. Similarly, *Bacteroidetes* levels fluctuate, with some studies showing increases and others decreases in both species. *Firmicutes* decrease in humans, while in mice, results are mixed, with both increases and decreases observed. Other phyla, including *Proteobacteria*, *Verrucomicrobia*, and *Tenericutes*, also exhibit inconsistent changes depending on the species and study. Overall, the microbiota shifts differently across species, with variations in findings (Sauer et al. 2021a) (Figure 1).

2.2 | Intestinal Barrier Permeability

In ASD individuals, disruptions in the intestinal barrier's integrity may lead to a phenomenon known as “leaky gut.” Multiple research studies have demonstrated impaired gut barrier function in individuals diagnosed with ASD. This increased permeability permits the migration of substances such as microbial toxins, undigested food particles, and pro-inflammatory cytokines from the gut lumen into the bloodstream, which may initiate immune responses and systemic inflammation. For example, individuals with ASD have been reported to exhibit alterations in tight junction proteins essential for maintaining the integrity of the intestinal barrier. Specifically, studies have found irregular expression levels of tight junction proteins such as occludin and zonulin in the intestines of individuals with ASD (Fasano 2012).

Also, some genetic factors can affect the structure and function of proteins involved in maintaining the integrity of the intestinal barrier. Variants in genes involved in tight junction proteins, mucus production, and immune function may contribute to increased susceptibility to intestinal permeability in people with ASD (Campbell et al. 2006; Sanders et al. 2015). Changes in tight junction proteins have been reported in several ASD models, including pre-natal zinc-deficient mice and *Shank3*$^{-/-}$ mice, where alterations in intestinal barrier integrity and inflammatory signaling were observed. These changes suggest a link between zinc deficiency, gut barrier dysfunction, and immune system abnormalities, potentially contributing to conditions

like ASD. The dysregulation of tight junction proteins like ZO-1 and E-cadherin highlights the impact of zinc deficiency on gut health and overall physiological processes (Sauer et al. 2021b) (Figure 1).

2.3 | Inflammation

The compromised barrier allows the passage of microbial agents and metabolites into the bloodstream, instigating inflammation by upregulating proinflammatory cytokine production. This systemic inflammation may induce proinflammatory processes in the developing brain of ASD individuals. This sequence of events encompasses heightened cytokine levels, microglial activation, and neuroinflammation. Individuals with ASD often have abnormalities in their immune response. Chronic inflammation and immune disorders can also affect and compromise the integrity of the intestinal barrier, exacerbating intestinal permeability. Immune cells and mediators can directly impact the tight junctions between intestinal epithelial cells, affecting their ability to maintain barrier function (Onore et al. 2012) (Goines and Ashwood 2013). For example, multiple studies have documented alterations in the immune system within the gut mucosa of individuals with ASDs (Buie 2015). These alterations involve dysregulation of innate and adaptive immune responses, such as changes in cytokine profiles, immune cell populations, and signaling pathways. Specifically, elevated levels of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), have consistently been reported in the intestinal mucosa of individuals with ASDs, indicating chronic inflammation within the gut microenvironment (Ashwood et al. 2011) (Figure 1).

Furthermore, abnormalities in immune cell populations, such as increased numbers of activated T cells and altered ratios of T helper cell subsets, have been identified in the gut mucosa of individuals with ASDs (Onore et al. 2012). These immune dysregulations suggest a complex interplay between immune dysfunction and gut pathology in ASDs.

The presence of GI symptoms in animal models for ASD generated by genetic and environmental manipulation raises the question of cause and consequence. It is possible that GI issues could arise from specific ASD behaviors, thus being the consequence of ASD and, therefore, present across various models displaying those characteristic behaviors. However, the gene products mutated in the genetic models for ASD are often expressed not only in the nervous system but also in the GI system and play a functional role there or are involved in regulating metal balance. Thus, their GI phenotype does not have to be secondarily caused by the ASD behaviors they show. It could be due to a converging regulatory role in the GI tract that is impacted by the presence of both mutations and certain environmental factors, such as metal imbalances.

Currently, several limitations must be overcome before conclusions regarding causality can be drawn. For example, whether gut pathologies occur after the onset of core behavioral ASD symptoms is difficult to establish as the diagnosis of ASD and also GI problems in very early childhood are problematic. Besides, while a gut pathology is caused by zinc deficiency, this does not exclude that zinc deficiency-specific brain pathologies also contribute to the ASD phenotype, such as SHANK and BDNF abnormalities (Grabrucker 2014; Koh et al. 2014; Yoo et al. 2016) Indeed, it is likely that several cerebral and extracerebral pathologies together contribute to causing ASD.

2.4 | Potential Links Between Microbiota Alterations, Immune Dysfunction, and Gut Pathology in ASDs

Research has emphasized the need to investigate the bidirectional connections between the immune system and gut microbiota, known as the gut-immune axis, in individuals with ASDs (Buie 2015). On the one hand, imbalances in the composition and function of the gut microbiota, called dysbiosis, can upset the immune balance and exacerbate gut inflammation in individuals with ASDs (Buie 2015). Disruptions in gut barrier function, resulting in increased intestinal permeability, may allow for the passage of microbial products and inflammatory mediators into the bloodstream, leading to ongoing immune activation and systemic inflammation. On the other hand, immune-mediated gut pathology, characterized by lymphocytes, mucosal inflammation, and alterations in epithelial barrier integrity, has been documented in ASDs, highlighting the intricate relationship between immune dysfunction and gut pathology (Ashwood and Van de Water 2004).

3 | Metal Imbalances in ASDs

Intriguingly, environmental factors, including diet, toxins, drugs, and infections, can affect intestinal permeability. For example, dietary factors such as gluten and casein have been hypothesized to worsen intestinal permeability in some people with ASD. Importantly, environmental toxins such as heavy metals can also disrupt intestinal barrier function (Genuis and Lobo 2014). Epidemiological data suggested a link between toxic heavy metals and the development of ASD, with lead (Pb), nickel (Ni), cadmium (Cd), and mercury (Hg) identified as potential contributors. These studies, primarily using hair, nails, and baby teeth samples, revealed higher levels of these metals in children with ASD compared to neurotypical controls (Błażewicz and Grabrucker 2022). For example, consistently, children with ASD have higher blood lead levels. The highest lead burden was reported in infants aged 0–3 years, highlighting the risk of early perinatal exposure (Yasuda and Tsutsui 2013). Despite some contradictory findings, a meta-analysis confirmed significantly higher lead concentrations in children with ASD (Saghazadeh and Rezaei 2017). Higher mercury levels have also been linked to ASD, especially in pre- and early postnatal periods, with primary teeth showing notably higher mercury amounts. Blood studies further supported these findings by indicating elevated mercury levels and increased biomarkers of mercury poisoning (Błażewicz and Grabrucker 2022).

The cellular pathologies seen in ASD, such as oxidative stress, mitochondrial dysfunction, and inflammation, are similar to those caused by heavy metal exposure. Heavy metals disrupt mitochondrial function, increasing oxidative stress, which is a characteristic feature of ASD. Mercury, for instance,

inhibits enzymes and proteins critical for antioxidant defense, exacerbating oxidative stress (Yin et al. 2007; Farina et al. 2011; Farina and Aschner 2019). Mitochondrial damage observed in children with ASD includes over-replication and deletions of mitochondrial DNA, lower oxygen consumption, and increased hydrogen peroxide production (Frye and Rossignol 2011). These mitochondrial issues align with ASD symptoms, suggesting a contributing role in ASD development (Napolioni et al. 2011). Heavy metals also influence gene expression related to oxidative stress and inflammation and can cause autoimmunity by increasing proinflammatory cytokines (Bjørklund et al. 2020; El-Fawal et al. 1999; Mishra 2009; Motts et al. 2014). Most importantly, they compete with essential metals like calcium and zinc, disrupting cellular signaling and metabolism.

Zinc deficiency is a recurring finding in children with ASD. Most studies report lower zinc levels in children with ASD, with the youngest showing the highest deficiency rates Babaknejad et al. (2016); Sayehmiri et al. (2015). A systematic review comparing Zinc levels between individuals with ASD and controls found that 36% of the included studies reported significantly lower zinc levels in ASD. At the same time, the remaining studies also indicated a trend toward reduced zinc concentrations do Nascimento et al. (2023). Another meta-analysis of 26 studies further confirmed that individuals with ASD had significantly lower blood zinc levels than controls, observed in 50% of the investigations Saghazadeh et al. (2017). Moreover, lower serum zinc levels and copper toxicity, characterized by a reduced zinc/copper ratio, were reported in children with ASD in the United States Faber et al. (2009), China Feng et al. (2023a), and Bangladesh Siddiqi et al. (2023). Notably, lower zinc/copper ratios correlated with higher scores on the Autism Behavior Checklist, indicating greater symptom severity Feng et al. (2023b). The correlation between ASD severity and the magnitude of zinc deficiency was also observed in other studies, where decreased serum zinc concentrations were associated with an increased risk and severity of ASD, suggesting a potential role for zinc deficiency in ASD pathophysiology Wu et al. (2022). However, some studies failed to find significant differences in plasma zinc levels between ASD and controls. These discrepancies may be influenced by geographical variations in zinc availability in soil and diet or by limitations associated with using plasma or serum zinc concentrations as biomarkers of zinc deficiency. Plasma zinc levels fluctuate due to fasting, transient dietary changes, circadian rhythms, and inflammation, making them less sensitive and specific indicators than whole blood zinc levels Roohani et al. (2013; Wieringa et al., 2015). Hair analysis has been suggested as a more reliable biomarker of long-term zinc status Lowe et al. (2009). In studies using hair samples, significantly lower zinc levels in children with ASD were confirmed Fiore et al. (2020; Yasuda et al. 2011), which again correlated negatively with ASD severity based on behavioral scales for play and creativity Fiore et al. (2020).

The relationship between various metal ions and ASD suggests that a unique metal profile, rather than a single metal deficiency or toxicity, might be linked to ASD. Recently, a model concept was proposed where changes in one metal trigger changes in other metals. Therefore, rather than a change in one trace metal, a unique and possibly characteristic metal profile may be linked to ASD. A toxic metal may increase ASD risk by interfering with zinc signaling on the cellular level. In contrast, zinc deficiency could increase the levels of toxic metals and exacerbate their effects (Błażewicz and Grabrucker 2022). These metals, like lead, can affect brain development directly and indirectly through mechanisms such as gut-brain signaling (Figure 2).

4 | The Role of Metals in Gut Pathology—A Systematic Review

To date, several narrative reviews and meta-analyses have explored the relationship between toxic metals and GI pathologies, toxic metals and ASD, as well as zinc deficiency and GI pathologies, and zinc deficiency and ASD independently. However, to our knowledge, a systematic approach aiming at a comparative analysis of the overlap of clinical GI symptoms reported in ASD and from exposure to toxic metals and zinc deficiency has not been performed. A systematic review has several advantages over a narrative review, primarily due to its structured and rigorous methodology, following a predefined protocol, including a detailed search strategy, inclusion/exclusion criteria, and data extraction process. Because of this, this approach helps reduce selection bias and publication bias, making systematic reviews the gold standard for evidence synthesis. Thus, comparing GI symptoms of individuals with toxic metal exposure, zinc deficiency, and ASD in a systematic review can help identify causative factors by ensuring a structured, unbiased, and comprehensive analysis of existing evidence, which helps establish biological plausibility supporting causation.

Therefore, the objective of the following systematic literature review is to elucidate whether metal imbalances may contribute to the underlying mechanisms of gut pathology in people with ASD. To that end, we provide an overview of various metals

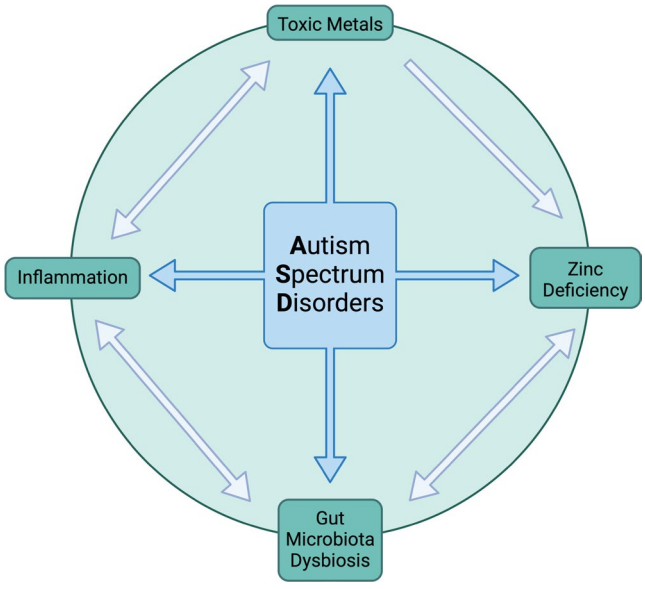

FIGURE 2 | Toxic metal exposure, inflammation, zinc deficiency, and gut microbiota dysbiosis are depicted as interconnected elements that could contribute to the development and progression of ASD. Created in BioRender. Grabrucker (2023) Created in BioRender. Grabrucker (2023) BioRender.com/b00f378.

present in the gut environment and their effects on gut pathology and differentiate between toxic and essential metals, particularly zinc, and their roles in gut health. A systematic review can establish consistent symptom patterns essential for identifying causative environmental factors by synthesizing high-quality evidence from multiple studies.

5 | Methodology

5.1 | Search Strategy

PubMed and Web of Science were searched from their inception to 2024 for studies regarding the association of microbiota changes and gut pathologies caused by cadmium, lead, mercury exposure, and zinc deficiency.

Pubmed and Web of Science search string (Heavy metal AND gut microbiome)

- "Mercury" AND "gut microbiome"
- "metal lead*" AND "gut microbiome"
- "Cadmium" AND "gut microbiome"
- "Zinc" AND "gut microbiome"
- "Zinc deficiency" AND "gut microbiome"

Pubmed and Web of Science search string (Heavy metal AND gut pathology)

- "Mercury" AND "gut pathology"
- "metal lead*" AND "gut pathology"
- "Cadmium" AND "gut pathology"
- "Zinc deficiency" AND "gut pathology"

*"metal lead" was chosen to avoid the heavy metal lead being mistaken for lead as in the verb "to lead".

Studies were then screened and excluded if the inclusion criteria below were not satisfied. Only original experimental research was included.

6 | Inclusion and Exclusion Criteria

6.1 | Inclusion Criteria

- Articles had to have human/murine studies. Any other species were excluded as those models were too far removed from humans.
- Comparative studies that included a control group.
- Only controlled heavy metal exposure studies were included

6.2 | Exclusion Criteria

- Letter, conference summary, case report, literature review, in vitro study
- Studies that looked at exposure to heavy metals other than mercury, cadmium, lead, and zinc, unless the study included one of these heavy metals, along with metals not being looked at in this review.
- Studies published in non-English languages.

Many of the gut pathologies observed in rodent studies have not been fully replicated in human studies due to the invasive nature of the required diagnostic procedures, which often involve surgical intervention or post-mortem examination. Therefore, we focused on rodent studies initially and compared the results with human study outcomes.

7 | Results

PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) criteria were applied for the systematic screening of relevant literature (Figure 3).

7.1 | Gut Pathologies in Rodent Models Caused by Toxic Metal Exposure

Several studies have investigated the effects of mercury on the GI system of rodents (Table 1; for a more detailed summary of results, see Table S1). The main findings can be summarized as mercury causing structural intestinal damage, increased intestinal barrier permeability and dysfunction, GI inflammation, and microbiota dysbiosis.

In addition, research studies examined the consequences of cadmium exposure for rodents' GI systems (Table 2; for a more detailed summary of results, see Table S2). The results can be summarized similarly as cadmium causing structural intestinal damage, increased intestinal barrier permeability and dysfunction, GI inflammation, and microbiota dysbiosis.

Furthermore, several studies have investigated the effects of lead on the GI system of rodents (Table 3; for a more detailed summary of results, see Table S3). Notably, the results show that lead also causes structural intestinal damage, increased intestinal barrier permeability and dysfunction, GI inflammation, and microbiota dysbiosis.

Therefore, from this analysis of pathologies taken from a systematic literature search, including studies that exposed rodents to these toxic metals, we conclude that the emerging pathologies are very similar between the different metals and may be caused by a common effect that these metals have, such as the generation of oxidative stress or the competition with essential divalent metals.

To better understand whether the latter point may be relevant, and given that zinc deficiency has been linked to ASDs, we next wanted to investigate whether a systematic literature search including studies that exposed rodents to zinc deficiency would yield the same array of GI pathologies (Table 4; for a more detailed summary of results, see Supplementary Table 4). Our results show that zinc deficiency in rodents causes structural intestinal damage, increased intestinal barrier permeability and dysfunction, GI inflammation, and microbiota dysbiosis. Therefore, the reported

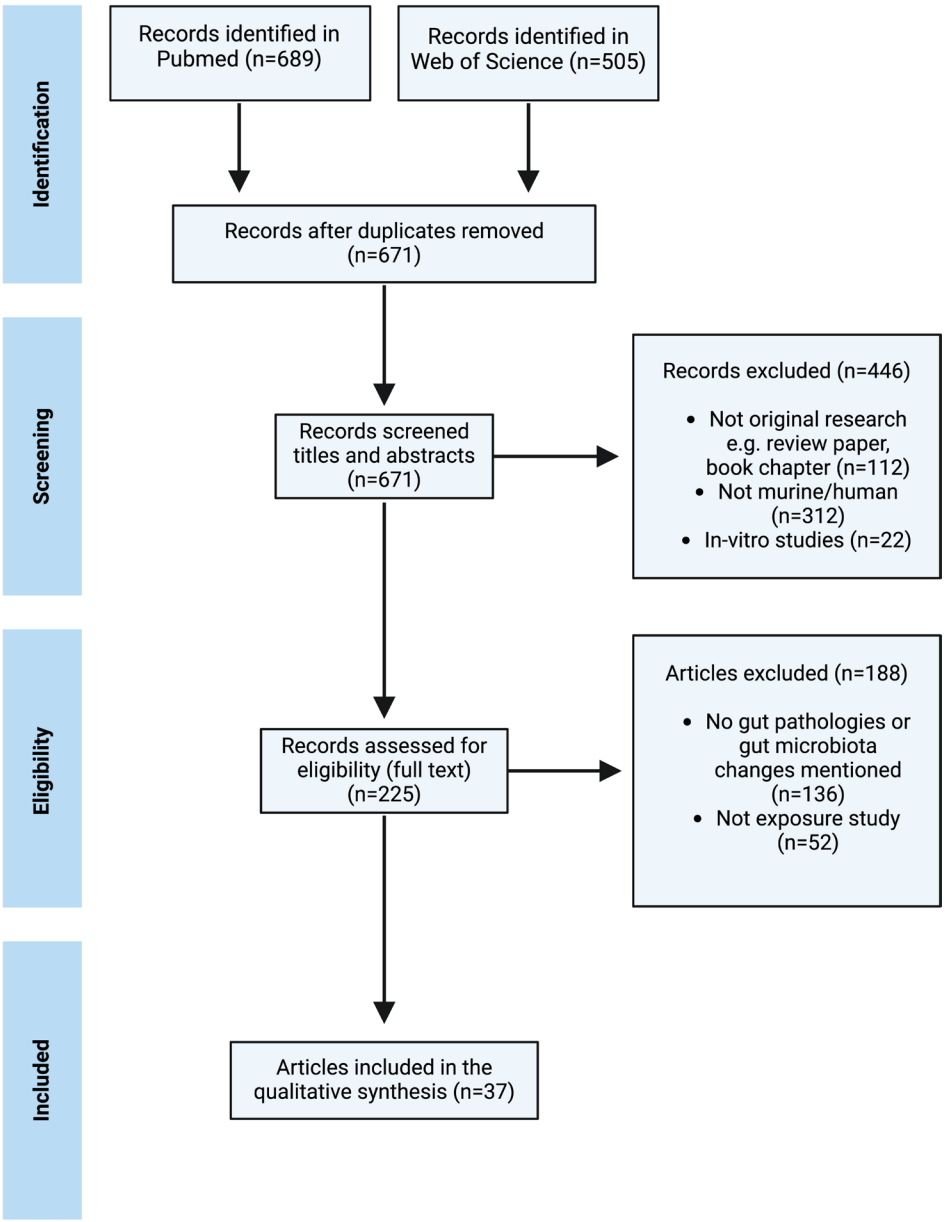

FIGURE 3 | PRISMA-based flow diagram depicting the study selection criteria and the number of studies included and excluded at each stage (Moher et al. 2009).

pathologies are the same as in studies with rodents exposed to lead, mercury, or cadmium. Thus, indeed, it is plausible that the presence of toxic metals reduces the bioavailability of zinc through competition with the essential metal for binding sites, i.e., on proteins (Figure 4). This mimics a situation of zinc deficiency.

These findings illustrate the overlapping effects of mercury (Hg), cadmium (Cd), lead (Pb), and zinc deficiency on intestinal health. For each toxic metal and zinc deficiency, the same specific aspects of intestinal pathology are reported, including intestinal barrier dysfunction: a breakdown in the protective lining of the intestines, allowing harmful substances to pass through; increased intestinal permeability, a condition where the intestinal lining becomes more permeable, allowing harmful substances and bacteria to enter the bloodstream; GI inflammation: this involves inflammation and irritation of the intestinal lining, leading to an increase in inflammatory cytokine production; and structural intestinal damage: this refers to physical damage to the intestinal tissue, such as thicker stomach walls, more inflammatory cells in the gastric mucosa, damage to intestinal villi and crypts, gut mucosa damage, changes in the architecture of villi, hemorrhagic gastritis, and intestinal epithelial cell necrosis.

7.2 | Gut Pathologies in Rodent Models Caused by Toxic Metal Exposure

The systematic review also included human studies. However, comparisons with rodent studies are limited as some analyses cannot be performed in humans. Especially, the availability of tissue samples hampers histological analysis of structural damage and intestinal barrier permeability. Besides, treatment of participants with toxic metals or induction of zinc deficiency is unethical. Therefore, the lengths and levels of exposure and deficiency

TABLE 1 | Summary of rodent studies investigating the association between mercury exposure, gut microbiota changes, and gut pathologies.

Author	Gut pathologies	Microbiota dysbiosis
(Yulan et al. 2020)	Intestinal barrier dysfunction	✓
(Natsumi et al. 2021)	No specific gut pathologies were mentioned; only microbiota dysbiosis	✓
(Nielsen et al. 2018)	Increased intestinal permeability	✓
(Ruan et al. 2019)	Structural intestinal damage	✓
(Xiaoying et al. 2021)	Structural intestinal damage Gut inflammation	✓
(Lin et al. 2020)	Gut inflammation	✓
(Lin et al. 2021)	Structural intestinal damage	✓

are much less controlled, as they occur naturally, for example, through diets deficient in zinc or living in contaminated areas near a mining and smelting site. In some studies, the causes of metal alterations are undetermined, and participants' metal status was measured to identify and group those with metal dysregulation.

Nevertheless, some studies met the inclusion criteria of our analysis (Table 5). In summary, these studies show that increased levels of mercury (Hg), cadmium (Cd), and lead (Pb) or decreased levels of zinc (Zn) result in microbiota dysbiosis, confirming observations in rodent models. In addition, GI pathologies such as impaired barrier integrity and increased inflammatory markers are reported, which are in line with rodent studies.

8 | Discussion

ASD is a complex neurodevelopmental condition characterized by a wide range of behavioral, social, and cognitive impairments. Beyond the hallmark symptoms of ASD, a significant subset of individuals with ASD experiences GI issues, leading to growing interest in understanding the connection between the gut and the brain in ASD. Both human studies and animal models, particularly rodent models, have been utilized to explore

TABLE 2 | Summary of rodent studies investigating the association between cadmium exposure, gut microbiota changes, and gut pathologies.

Author	Gut pathologies	Microbiota dysbiosis
(He et al. 2020a)	Increased intestinal permeability Gut inflammation	✓
(Zhang et al. 2023)	No specific gut pathologies were mentioned; only microbiota dysbiosis	✓
(Zhang et al. 2015)	Structural intestinal damage	✓
(Li et al. 2020)	Structural intestinal damage	✓
(Yang et al. 2024)	No specific gut pathologies were mentioned; only microbiota dysbiosis	✓
(Ba et al. 2017)	No specific gut pathologies were mentioned; only gut microbiota dysbiosis	✓
(Li et al. 2022a)	Gut inflammation Structural intestinal damage	✓
(Zhao et al. 2006)	Gut inflammation Structural intestinal damage	No specific gut microbiota findings
(Breton et al. 2016)	Gut inflammation	✓
(He et al. 2020a)	No specific gut pathologies mentioned	✓
(Yue et al. 2023)	Increased intestinal permeability	✓
(Wu et al. 2023)	No specific gut pathologies mentioned	✓
(Dai et al. 2022)	Gut inflammation Reduced butyrate production	✓
(Yang et al. 2021)	Gut inflammation	✓
(Ninkov et al. 2015)	Structural intestinal damage	✓
(Ninkov et al. 2016)	Structural intestinal damage	✓

TABLE 3 | Summary of rodent studies investigating the association between lead exposure, gut microbiota changes, and gut pathologies.

Author	Gut pathologies	Microbiota dysbiosis
(Gao et al. 2017)	No specific gut pathologies were mentioned; only microbiota dysbiosis	✓
(Hu, Xiao et al. 2023)	Structural intestinal damage Gut inflammation	✓
(Shen et al. 2024)	Increased intestinal permeability	✓
(Wang et al. 2024)	Structural intestinal damage	✓
(Sadykov et al. 2009)	No specific gut pathologies were mentioned; only gut microbiota dysbiosis	✓
(Chen et al. 2022)	No specific gut pathologies were mentioned; only gut microbiota dysbiosis	✓
(Yu et al. 2021)	Structural intestinal damage Gut inflammation	✓
(Breton et al. 2013)	Gut inflammation	No specific gut microbiota findings
(Yang et al. 2023)	Gut inflammation	✓

TABLE 4 | Summary of rodent studies investigating the association between zinc/zinc deficiency exposure, gut microbiota changes, and gut pathologies.

Author	Gut pathologies	Microbiota dysbiosis
(Davis 2nd et al. 2022)	Zinc deficient group: Gut inflammation	No specific gut microbiota findings
(Foligné et al. 2020)	Zinc deficient group: Gut inflammation, diarrhea, and structural intestinal damage	No specific gut microbiota findings
(Sauer and Grabrucker 2019)	Zinc deficient group: Intestinal barrier dysfunction, gut inflammation, and increased intestinal permeability	✓
(Sauer et al. 2021a)	Zinc deficient group: Intestinal barrier dysfunction, gut inflammation, and increased intestinal permeability	✓
(Zhong et al. 2013)	Zinc adequate diet plus ethanol diet and zinc deficient diet plus ethanol = increased intestinal permeability	No specific gut microbiota findings

the gastrointestinal pathologies associated with ASD, shedding light on potential mechanisms.

This discussion is based on a systematic analysis of original literature, not a summary of previous review articles, providing a novel perspective on the interplay between metal dysregulation, GI pathologies, and ASD. Several hypotheses have been proposed to explain the high prevalence of GI issues in individuals with ASD: The gut-brain axis, a bidirectional communication pathway between the central nervous system (CNS) and the GI system, is thought to be disrupted in ASD. This could result in altered gut motility, increased intestinal permeability ("leaky gut"), and microbiota dysbiosis (Yu et al. 2024). Ultimately, this may result in immune system abnormalities, including increased levels of pro-inflammatory cytokines noted in ASD patients (Suprunowicz et al. 2024). This could contribute to inflammation in the GI tract, leading to symptoms like diarrhea and abdominal pain. In addition, specific gut microbiome alterations, such as reduced diversity and increased levels of certain microbial species, may contribute to GI dysfunction and potentially exacerbate behavioral symptoms through microbial metabolites affecting brain function (Peralta-Marzal et al. 2021).

Rodent models have been extensively used for detailed mechanistic studies and the evaluation of therapeutic interventions. Several genetic and environmental models of autism exhibit GI abnormalities that parallel findings in human patients. For example, mice modeling genetic risk factors for ASD, such as *Shank3*, *Cntnap2*, and *Fmr1* knockout models, exhibit both behavioral and GI symptoms (Altimiras et al. 2021; Robinson et al. 2023; Sauer et al. 2019; Tabouy et al. 2018). For instance, *Shank3* knockout mice display reduced intestinal motility, increased intestinal permeability, and altered gut microbiota composition (Sauer et al. 2019; Tabouy et al. 2018). This mirrors the constipation and dysbiosis seen in human patients with ASD.

Besides, mice modeling non-genetic risk factors for ASD, such as mice with prenatal exposure to valproic acid (VPA), which induces ASD-like behaviors in rodents, also display GI abnormalities (Liu et al. 2018; Sauer et al. 2022). VPA-treated rodents exhibit increased gut permeability, inflammation, and

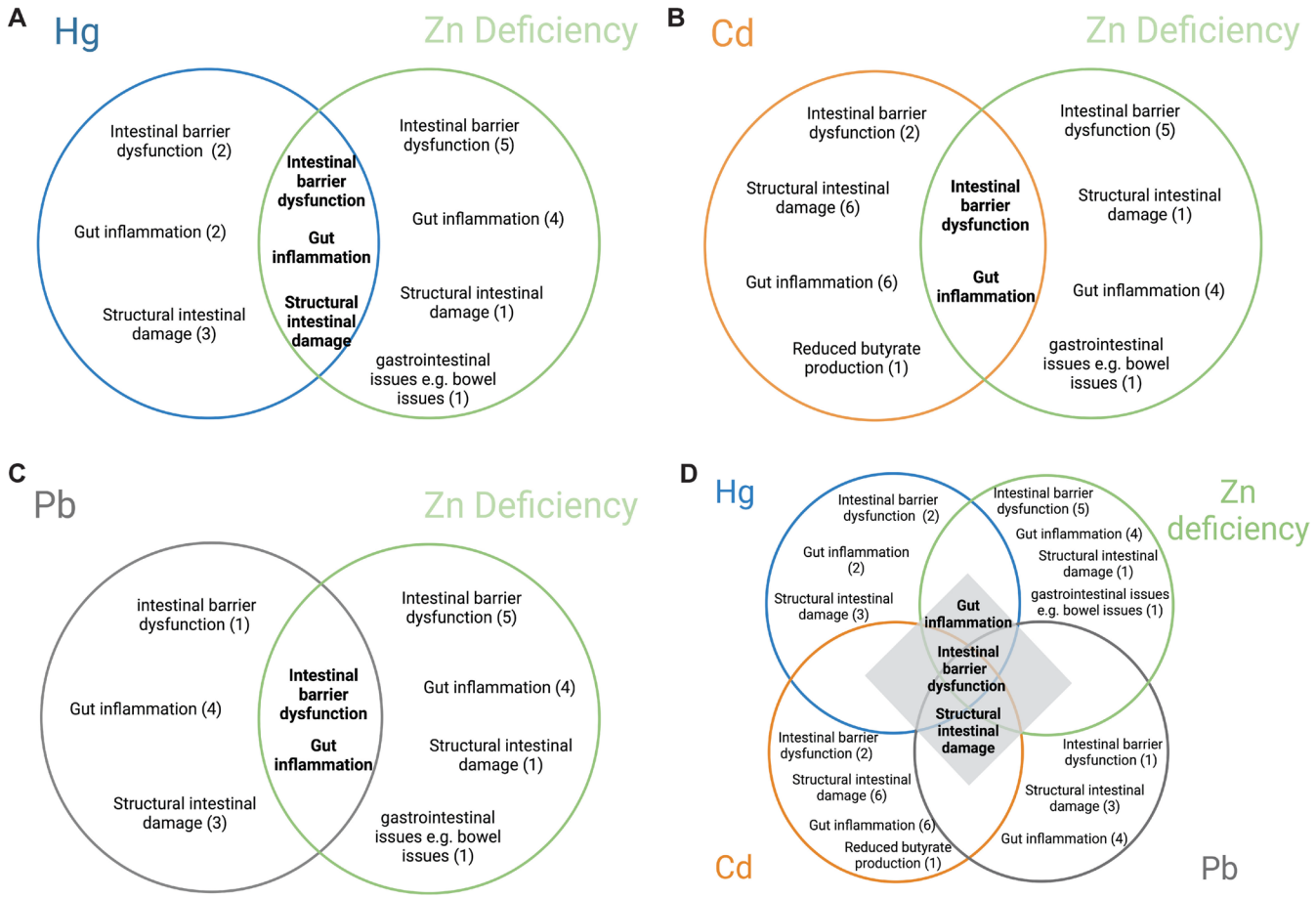

FIGURE 4 | Interconnected Effects of toxic metals and zinc deficiency and their impact on GI health. (A) Venn diagram illustrating the overlapping effects of mercury exposure and zinc deficiency on intestinal function. Both factors can contribute to intestinal barrier dysfunction, increased intestinal permeability, gut inflammation, and structural intestinal damage. (B) Venn diagram illustrating the overlapping effects of cadmium exposure and zinc deficiency on intestinal function. Both factors can contribute to intestinal barrier dysfunction, increased intestinal permeability, gut inflammation, and structural intestinal damage. (C) Venn diagram illustrating the overlapping effects of lead exposure and zinc deficiency on intestinal function. Both factors can contribute to intestinal barrier dysfunction, increased intestinal permeability, gut inflammation, and structural intestinal damage. (D) Overlapping effects of mercury (Hg), cadmium (Cd), lead (Pb), and zinc deficiency on intestinal health. The Venn diagram illustrates the interconnected pathways through which these factors can contribute to intestinal barrier dysfunction, increased intestinal permeability, gut inflammation, and structural intestinal damage. (A–D) The numbers within each section indicate the number of studies supporting the corresponding effect.

microbiota shifts. In addition, mice exposed to maternal immune activation (MIA), another commonly used model for ASD, show an increased abundance of certain bacterial species, including *Akkermansia muciniphila*, which has been linked to gut inflammation and altered behavior (Tartaglione et al. 2022). Furthermore, prenatal zinc deficiency, which induces ASD-like behaviors in rodents, also induces GO pathologies such as increased gut permeability, inflammation, and microbiota shifts (Sauer et al. 2021b).

Studies using rodent models have shown that gut microbiota can directly affect behavior. For example, transplanting gut microbiota from ASD patients into mice has been shown to induce both GI symptoms and behavioral changes reminiscent of ASD, further supporting a causal link between gut microbiota and the brain in ASD (Li et al. 2022b; Wang et al. 2023). Besides, Fecal Microbiota Transplantation relieved GI and ASD Symptoms in an open-label study in humans (Li et al. 2021).

Interestingly, the specific findings in rodent ASD models can be summarized as follows:

A. Increased intestinal permeability. Several rodent models of ASD show evidence of a "leaky gut," where the intestinal barrier is compromised, allowing larger molecules, such as lipopolysaccharides (LPS), to pass through and potentially trigger systemic inflammation.

B. Microbiota Dysbiosis: As in human studies, rodent models consistently exhibit alterations in gut microbiota.

C. GI dysfunction, such as gut motility issues: Alterations in gut motility, such as delayed transit times, have been documented in rodent models.

These findings are very consistent with our findings on rodents exposed to toxic metals and zinc deficiency. It is plausible that toxic metals, in competition with zinc, lower the bioavailability of zinc, thereby re-creating the pathology observed in

TABLE 5 | Summary of human studies investigating the association between toxic metals/zinc deficiency exposure, gut microbiota changes, and gut pathologies.

Authors	Metal dysregulation	Gut pathologies	Microbiota changes
(Eggers et al. 2023)	The average levels of Pb exposure measured in maternal whole blood during the second and third trimesters of pregnancy	Gut microbiota alterations Potential alterations in gut barrier integrity due to metal-associated shifts in the gut microbiome	Specific microbiota alterations associated with prenatal Pb exposure: 6 bacterial taxa were negatively associated with second-trimester Pb exposure, and 3 bacterial taxa were negatively associated with third-trimester Pb exposure
(Han et al. 2015)	Subjects with high Cd through contaminated food, water, and air	Metabolic syndrome, inflammation, and altered metabolism of short-chain fatty acids (SCFAs): decrease in SCFA. Gut microbiota alterations	Fewer butyrate-producing bacteria due to Cd exposure
(Zhai et al. 2019)	Hg, Pb, As, Cu, Zn, Fe, Ca, and Mg in hair samples of Chinese children with ASD	GI dysfunction: abdominal pain, constipation, diarrhea, bloating. Gut microbiota alterations. Positive correlation between ASD severity and GI disorders	High Pb or high Hg is significantly associated with the abundance of Parabacteroides and Oscillospira
(Shao and Zhu 2020)	As, Cd, Cu, Pb, and Zn exposure through living in contaminated areas near a mining and smelting site	Gut microbiota alterations	Pb exposure: Increased Lachnospiraceae, *Eubacterium eligens*, Ruminococcaceae UGG-014, Erysipelotrichaceae UCG-003, Tyzzerella 3, Bacteroides, Slackia, and Roseburia. Pb exposure: Changes in Bacteroides, Roseburia, Prevotella 9, and depletion of Proteus
(Chai et al. 2024)	Participants with dietary zinc deficiency (ZD)	Metabolic syndrome, inflammation, and potential barrier dysfunction (saccharin, the pro-inflammatory metabolites, and taurocholic acid, the potential factor inducing intestinal leakage, were higher in the ZD group)	ZD group had a significantly increased Phocaeicola vulgatus, *Alistipes putredinis*, *Bacteroides uniformis*, Phocaeicola sp000434735, and *Coprococcus eutactus*, but a decrease in the probiotic bacteria *Bifidobacterium kashiwanohense*

prenatal and acute zinc-deficient mice (Sauer et al. 2019; Sauer et al. 2021a).

The absorption and regulation of zinc in humans and rodents are mediated by two transporter families: the zinc importer family (ZIP) and the zinc transporter family (ZnT). Among these, ZIP4, localized on the apical membrane of enterocytes, is the primary transporter responsible for zinc uptake from the intestinal lumen (Roohani et al. 2013). Consequently, intestinal zinc absorption primarily occurs through a carrier-mediated transport mechanism. In response to zinc deficiency, ZIP4 expression is upregulated in the intestine (Hashimoto et al. 2016). However, this compensatory mechanism also increases the uptake of toxic metals, such as cadmium (Cd) and lead (Pb). Therefore, zinc levels exhibit a significant inverse association with serum Cd concentrations (Vance and Chun 2015).

Once inside the enterocytes, metallothionein is a transient zinc storage protein. The release of zinc into the portal circulation is primarily regulated by ZnT1, which is localized on the basolateral membrane of enterocytes and pancreatic acinar cells. ZnT1 is the main efflux transporter, facilitating zinc transport from intestinal epithelial cells into the bloodstream (Cousins 2010). Several hormonal and cytokine-mediated mechanisms regulate systemic and cellular zinc homeostasis. For instance, the pro-inflammatory cytokine interleukin-1β (IL-1β) has been shown to downregulate zinc transporters in pancreatic islet cells (Egefjord et al. 2009; El Muayed et al. 2010). Thus, infections can disrupt zinc equilibrium, leading to an intracellular zinc shift that supports protein synthesis and neutralizes free radicals (Gammoh and Rink 2020). This may explain the increased risk for ASD in women with infections during pregnancy.

Several metal ions, including zinc, are essential for the survival of microorganisms, as they serve as cofactors for numerous enzymes and are required for various biological processes. Metabolic demands drive the uptake of metal ions by bacteria, and any disruption in metal homeostasis can harm bacterial viability (Porcheron et al. 2013). Thus, zinc is vital for gut commensal microbiota and crucial in maintaining microbial balance. Some pathogenic bacteria, such as *Salmonella enterica*, can utilize zinc chelation to outcompete host-associated commensal bacteria, which are less adapted to zinc-restricted environments within the inflamed gut (Liu et al. 2012). This mechanism highlights the importance of zinc availability in the gut as a key factor influencing competitive interactions among different bacterial species using specialized metal uptake systems. While some gut commensals possess metal transporters that enable them to thrive under zinc-deficient conditions, others are significantly affected in viability. This suggests that zinc availability significantly impacts the balance between bacterial populations, ultimately affecting GI physiology.

Therefore, zinc is essential for microbiota diversity. Its deficiency is implicated in various GI diseases and contributes to intestinal hyperpermeability, allowing toxins and bacterial antigens to enter the bloodstream (Skrovanek et al. 2014; Xia et al. 2021). The underlying mechanism is the zinc-dependent regulation of tight junction (TJ) complexes, maintaining epithelial integrity by preventing unregulated paracellular exchange. Disruptions in zinc homeostasis weaken TJ function, increasing gut permeability and inflammation (Sauer et al. 2021a; Ulluwishewa et al. 2011).

Altered gut microbiota is frequently reported in ASD, with leaky gut linked to pro-inflammatory responses and neurodevelopmental changes via the gut-brain axis (Fiorentino et al. 2016). ASD is also associated with reduced probiotic bacteria, overgrowth of pathogens, and increased inflammatory cytokines, exacerbating symptoms. A meta-analysis of available human studies found that children with ASD had significantly lower zinc levels and altered microbiota composition, including decreased Bifidobacterium and Parabacteroides alongside increased Bacteroides and Clostridium (Lin et al. 2023). While limited human studies have explored the zinc-microbiota-gut-brain connection, in animal studies, zinc deficiency is indeed causative for altered microbiota diversity and, during pregnancy, may influence both the maternal microbiota and offspring gut microbiota composition, triggering inflammation, including neuroinflammation, and resulting in ASD behaviors (Sauer and Grabrucker 2019).

Our findings, derived from an original systematic literature analysis, provide novel insights into the role of metal dysregulation in shaping GI pathologies and their potential impact on ASD. These findings suggest a possible mechanism whereby metal imbalances, particularly zinc deficiency, or metal imbalances and biological processes such as toxic metal overload and infections creating a secondary zinc deficiency during GI development may contribute to GI abnormalities observed in ASD. Still, more importantly, they prove that core pathological processes in the GI are zinc-dependent, which has significant implications for prevention and therapeutic strategies. Similarly, they emphasize the importance of considering metal homeostasis as a crucial factor in the pathophysiology of ASD. They also highlight the potential for developing novel therapeutic strategies that target metal imbalances to improve GI health and potentially alleviate ASD symptoms.

Although this review investigated the overload of toxic metals, it should also be mentioned that other essential metals, particularly copper, can compete with zinc for absorption and biological processes (Grabrucker 2023). Thus, some essential heavy metals can have adverse effects when consumed in excess, leading to toxic outcomes and disrupting normal biological processes. For example, a mouse model for copper overload showed secondary zinc deficiency that impacted the zinc-dependent ASD-linked proteins of the SHANK family (Baecker et al. 2014). An increased copper/zinc ratio has also been linked to ASD in human studies (Bjorklund 2013). Copper levels and ceruloplasmin concentrations were found to be significantly higher in children with ASD, with a significant association between ASD and both zinc deficiency and copper toxicity (Siddiqi et al. 2023).

8.1 | Conclusions

GI pathologies are a significant concern in individuals with ASD and appear to play a role in both the physical and behavioral aspects of the disorder. Estimates suggest that 30% to 70% of

children with ASD suffer from some form of GI disturbance, far exceeding the rates observed in the general pediatric population. These symptoms often correlate with the severity of ASD, with patients exhibiting more severe behavioral symptoms tending to report more significant GI discomfort (Al-Beltagi et al. 2023; Valicenti-McDermott et al. 2006). Rodent models have proven invaluable in unraveling the complex mechanisms underlying these GI issues, highlighting the role of microbiota, immune dysregulation, and gut-brain axis disturbances. However, it seems that these mechanisms are highly metal-dependent. Thus, metal imbalances in ASD warrant more research attention due to their potential role in the pathophysiology of the disorder and their influence on neurodevelopmental processes. Metals such as zinc, copper, and iron are essential cofactors in enzymatic reactions critical for brain development, synaptic function, and antioxidant defense. Imbalances, particularly elevated levels of toxic metals like lead or mercury and altered ratios of essential metals like zinc to copper, have been observed in some individuals with ASD. However, the precise mechanisms linking metal dysregulation to ASD symptoms are not fully understood, and inconsistent findings across studies highlight the need for more rigorous, large-scale investigations. Continued research into metal dyshomeostasis in ASD holds promise for the development of targeted therapies that could improve the quality of life for individuals with ASD by addressing both GI and core behavioral symptoms. In conclusion, based on a comprehensive systematic analysis of original research, this study provides novel insights into the critical role of metal dyshomeostasis in the pathophysiology of ASD. These findings extend beyond previous reviews by directly examining original research data, highlighting the significant impact of metal imbalances on GI function and their potential contribution to ASD symptomology by systematically revealing the substantial overlap in GI symptoms between exposure to toxic metals, zinc deficiency, and ASD.

9 | Future Directions and Considerations

Our analysis has shown that several factors discussed as causative for ASD have converging organ, tissue, and cellular pathologies. Toxic metals, in particular, seem to reproduce a local zinc deficiency. On the other hand, this confirms that the pathologically relevant mechanisms in the GI tract in ASD are zinc-dependent. This may open new vistas for new research, as well as preventive and therapeutic strategies.

9.1 | Suggestions for Further Research

Research on metal imbalances related to gut health is an emerging and complex field. A multifaceted approach combining basic, clinical, and translational research is essential to unravel the complexities of metal imbalances and their effects on GI health. For example, through interdisciplinary collaborations between microbiologists, gastroenterologists, nutritionists, toxicologists, and other experts, further investigation into how zinc influences gut microbiota and how imbalances contribute to GI health and disease should be performed. This includes understanding how different metals interact with specific microbial species, the metabolic pathways involved, and how they interact with the host GI tissue. Future research should investigate the bidirectional relationship between metals and microbiota, that is, how the gut microbiome influences metal absorption, storage, and metabolism, and how metals influence gut microbiota behavior and composition, that is, the genetic and functional diversity of gut microbiota as a result of metal metabolism.

Furthermore, identifying reliable biomarkers for metal imbalances that can be used for early diagnosis, monitoring of GI health, and markers for effective dietary and pharmaceutical interventions to correct metal imbalances needs to be proposed. This will facilitate clinical trials to assess the effects of metal supplementation or depletion in patients with GI-related diseases such as IBD and GI problems in ASD. These interventions, among others, may include exploring nutraceuticals, functional foods, and probiotics, prebiotics, and synbiotics tailored to modulate metal levels and improve gut health.

Besides, large-scale epidemiological studies to explore the prevalence of metal imbalances in different populations, including individuals with ASD, and their association with gut health outcomes will help identify at-risk groups and inform treatment strategies.

9.2 | Therapeutic Interventions

The overlapping GI pathologies in rodent models and human patients with ASD suggest potential therapeutic targets aimed at the gut-brain axis. Current research is exploring several avenues for treatment:

Nutraceutical interventions show promise as preventive and therapeutic strategies for ASD, with a focus on addressing metabolic imbalances and supporting synaptic protein function. Supplements like vitamins and minerals may help alleviate symptoms and enhance the effectiveness of behavioral therapies (Bakkaloglu et al. 2008). Many children with ASD experience nutritional deficiencies due to inadequate intake, gastrointestinal issues, and disrupted transport of vitamins across the blood–brain barrier (Bakkaloglu et al. 2008). Research suggests that prenatal use of multivitamins can reduce the risk of ASD, highlighting the importance of proper nutrition during critical developmental periods (Arking et al. 2008).

Vitamins B1, B5, B6, and D are involved in key processes like neurotransmitter production, synaptic regulation, and metabolic activity. Their supplementation has shown benefits for managing ASD symptoms, improving behaviors, and supporting neural function (Laumonnier et al. 2004; Parmeggiani et al. 2018; Poulopoulos et al. 2009; Walker and Scherer 2013).

Probiotics and Prebiotics: Modulating gut microbiota through probiotics or prebiotics has been shown to alleviate some GI symptoms and improve behavioral outcomes in rodent models (Feng et al. 2023a; Soleimanpour et al. 2024; Zhang et al. 2024). However, while human and animal studies consistently show differences in microbiota composition between the control and ASD populations, no clear trend regarding a specific bacterial profile characteristic of ASD has emerged. Thus, targeted interventions are hampered by the lack of a defined microbiota composition that needs to be achieved.

Therefore, although these nutraceuticals show potential to improve both ASD-related symptoms and associated conditions, no supplements have been officially approved for ASD treatment. Customized combinations of these interventions may enhance benefits, but further clinical trials are necessary to confirm their effectiveness.

Anti-inflammatory Therapies: Given the role of immune dysregulation and gut inflammation, anti-inflammatory treatments are also being investigated in rodent models and human patients. Reducing inflammation in the gut may help alleviate both GI and behavioral symptoms in ASD (Arteaga-Henríquez et al. 2023; Singh et al. 2023).

Dietary Interventions: Gluten-free and casein-free diets, along with the use of specific fibers to promote beneficial bacterial growth, have been explored in both rodent models and human studies (Alam et al. 2022; Alsubaiei et al. 2023). While results are mixed, these interventions hold promise for a subset of ASD patients, particularly those with marked GI symptoms. However, based on the presented results, metal homeostasis should be a key therapeutic target in ASD.

Several research studies have investigated the effects of zinc supplementation on gut health. These studies suggest that zinc aids in maintaining the integrity of the GI tract and modulating immune responses within the gut. For example, zinc supplementation has been found to enhance intestinal barrier function, reducing permeability in a variety of pathologies, including diarrhea, inflammatory bowel diseases (IBD), other GI ailments, and even some neurological conditions (Skrovanek et al. 2014), and preventing the translocation of harmful substances from the gut into the bloodstream. Thus, zinc supplementation is frequently discussed for IBD (Ananthakrishnan et al. 2015; Chao 2023; Hu, Zhao et al. 2023; Sakurai et al. 2022; Sturniolo et al. 2001), as zinc has been shown to exert anti-inflammatory effects in the gut, reducing the production of pro-inflammatory cytokines and promoting the activity of anti-inflammatory pathways.

Additionally, zinc supplementation has been linked to improved gut microbiota composition (Usama et al. 2018). It promotes the growth of beneficial bacteria while inhibiting the proliferation of pathogenic organisms (Xia et al. 2021). This balance in the gut microbiome is crucial for overall digestive health and immune function. In ASD, almost no clinical trials have been performed. However, zinc supplementation was promising in a study with 79 autistic individuals. Autistic children with GI pathology significantly improved concerning hyperactivity after zinc therapy, while autistic children without GI disease did not improve (Russo 2011).

While the current approaches are still inconclusive, having identified a strong association between GI issues in ASD and metal imbalances opens new treatment possibilities that are currently not well explored, which makes us optimistic. Overall, the findings from these research studies suggest that zinc supplementation holds promise as a therapeutic intervention for improving gut health and managing GI disorders, potentially including GI issues as co-morbidity of ASD. Zinc has also demonstrated potential by strengthening synaptic scaffolding proteins, such as SHANK3, and mitigating inflammation in preclinical models (Ching et al., 2010; Dabell et al., 2013; Girirajan et al., 2013; Yan et al., 2005; Zhang et al. 2023). However, further research is needed to fully elucidate the optimal dosages and treatment protocols. Notably, research studies investigating metal chelation therapy for gut health have also shown promising results, particularly in conditions associated with metal toxicity or dysbiosis (Bamonti et al. 2011). Metal chelation therapy involves using chelating agents to bind and remove excess specific metals from the body, thereby reducing their toxic effects. This approach has been explored in the gut primarily for conditions such as IBD (Bamonti et al. 2011). For example, studies have demonstrated that metal chelators, such as EDTA (ethylenediaminetetraacetic acid) and DMSA (dimercaptosuccinic acid), can effectively reduce levels of toxic metals like lead, mercury, and cadmium in the gut. By lowering their burden, these therapies alleviate inflammation, oxidative damage, and barrier dysfunction in the intestinal mucosa (Crichton 2016).

Like zinc supplementation, metal chelation therapy can modulate the gut microbiota composition, promoting beneficial bacteria growth while suppressing pathogenic species' proliferation (Duan et al. 2020). However, while promising, it is essential to note that metal chelation therapy may also have potential side effects, such as nutrient depletion and disruption of essential metal homeostasis (Flora and Pachauri 2010). Therefore, long-term efficacy and safety profiles must be established, as more adverse effects than zinc supplementation are expected.

Author Contributions

Katelyn O'Grady: writing – original draft, writing – review and editing, formal analysis, data curation. **Andreas M. Grabrucker:** conceptualization, writing – review and editing, supervision, resources.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the Supporting Information of this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.