Chapter

Exposure to Xenobiotics and Gene-Environment Interactions in Autism Spectrum Disorder: A Systematic Review

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Abstract

Heritability estimates indicate that genetic susceptibility does not fully explain Autism Spectrum Disorder (ASD) risk variance, and that environmental factors may play a role in this disease. To explore the impact of the environment in ASD etiology, we performed a systematic review of the literature on xenobiotics implicated in the disease, and their interactions with gene variants. We compiled 72 studies reporting associations between ASD and xenobiotic exposure, including air pollutants, persistent and non-persistent organic pollutants, heavy metals, pesticides, pharmaceutical drugs and nutrients. Additionally, 9 studies reported that interactions between some of these chemicals (eg. NO_2, particulate matter, manganese, folic acid and vitamin D) and genetic risk factors (eg. variants in the *CYP2R1*, *GSTM1*, *GSTP1*, *MET*, *MTHFR* and *VDR* genes) modulate ASD risk. The chemicals highlighted in this review induce neuropathological mechanisms previously implicated in ASD, including oxidative stress and hypoxia, dysregulation of signaling pathways and endocrine disruption. Exposure to xenobiotics may be harmful during critical windows of neurodevelopment, particularly for individuals with variants in genes involved in xenobiotic metabolization or in widespread signaling pathways. We emphasize the importance of leveraging multilevel data collections and integrative approaches grounded on artificial intelligence to address gene–environment interactions and understand ASD etiology, towards prevention and treatment strategies.

Keywords: autism spectrum disorder, xenobiotic exposure, early-life exposure, genetic risk factors, gene-environment interactions, exposome

1. Introduction

Many neuropsychiatric disorders are thought to have a multifactorial etiology, with interactions between genetic susceptibility and environmental factors likely contributing to their onset and progression [1]. ASD has a particularly complex genetic architecture, with implicated genes accumulating thanks to more accessible and less costly high-throughput genotyping and sequencing technologies. Between 15 to 25% of ASD cases occur in the context of clinically defined monogenic syndromes and chromosomal rearrangements [2], and therefore have a genetic

diagnosis. However, most patients still do not have a clearly identified genetic cause. Genome-wide association studies (GWAS), carried out in large cohorts using SNP arrays, did not find consistently associated ASD genes [3], but showed that individuals with ASD carry a significantly higher burden of *de novo* Copy Number Variants (CNVs) than expected [4, 5]. More recently, exome and genome sequencing studies have been detecting a growing number of loss-of-function Single Nucleotide Variants (SNVs) in patients [5, 6]. Some of these SNVs are rare *de novo* genetic variants with high penetrance, but most have low to moderate effects, indicating that a multiplicity of common, low effect variants are discrete contributors to ASD risk variance. These CNVs and SNVs map to dozens of different candidate genes, which frequently cluster in neurobiological pathways (*e.g.* synaptic processes, behavior regulation, cognition and neuronal signaling) as well as in chromatin modification and gene expression regulation processes [4–7], providing evidence for the biological mechanisms disrupted in the disorder.

Recent ASD heritability estimates vary between 64 and 85% [8, 9], and incomplete concordance rates between monozygotic twins are reported [10, 11]. These observations suggest that ASD, and its hallmark clinical heterogeneity, is not solely determined by genetics, and that environmental factors may contribute to its risk. Due to the extreme vulnerability of the developing brain to environmental stressors [12], the impact of environmental factors in this neurodevelopmental pathology is of particular concern. In this context, the environment comprises all non-genetic factors that can influence the onset or progression of the disease. Generally, environmental factors include xenobiotics, *i.e.* any natural or synthetic foreign agent that enters the organism through ingestion, inhalation, dermal absorption, injection or by placental transfer, and also other external factors like medical events or lifestyle, psychosocial and cultural variables [13, 14].

From conception to death, individuals are to some degree shaped by an ever-changing environment. However, its impact in health and disease through the life course is still mostly unexplored. Given the early onset of ASD, environmental exposure during the prenatal period to the second year of life is of particular relevance, while at later stages it may still modulate disease progression and possibly treatment efficacy [13, 15]. In this review we focus specifically on the role of xenobiotics in ASD, and on the impact of interactions between genetic variants and xenobiotic exposure. Literature reporting xenobiotic exposure in ASD is already extensive. We expect this systematic review may guide and encourage further studies to elucidate the impact of gene–environment interactions in ASD.

2. Methods

We systematically reviewed studies in two categories: (a) studies reporting xenobiotic exposure implicated in ASD; (b) studies reporting interactions between the previously defined xenobiotics and any genetic factor. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standard checklist [16]. Systematic reviews of the literature were performed successively for categories (a) and (b).

2.1 Information sources and search strategy

PubMed and EBSCO were queried from inception to November 2020, for records published in peer-reviewed English-language journals.

For records in category (a) PubMed and EBSCO were interrogated using updated and dropped clinical terms (“autis*”; “asperger” and “pervasive

developmental disorder") in combination with the terms "environment*", or "xenobiotic", or "toxin" or with terms for xenobiotics' names ("antidepressants"; "air pollutants"; "bisphenol A"; "folic acid"; "metal"; "PBDE"; "PCB"; "pesticide"; "PFC"; "phthalate"; "vitamin D"). Regarding category (b) the query was done using the same clinical terms in combination with "gene–environment" term and with terms for xenobiotics names identified in previous search.

2.2 Screening and eligibility criteria

All identified records were imported to the Mendeley reference manager. PRISMA flowcharts for (a) and (b) categories are shown in **Figure 1**. For record screening, the following exclusion criteria were applied: 1) review articles and letters to editor; 2) articles where the participants' diagnosis of ASD was not confirmed according to criteria from *The Diagnostic and Statistical Manual of Mental Disorders III, IV* or *5* editions or from the *International Classification of Diseases 9* or *10* editions; 3) articles not related to exposure to xenobiotics (category (a)) or not related to gene–environment interactions (category (b)); 4) articles focusing only on animal models, because despite the existence of several robust animal models that provide insight into the biological mechanisms and therapeutics for the disorder, these are unable to fully comprise the behavioral spectrum; 5) articles reporting associations between vaccination or thimerosal exposure and ASD (category (a)), because a role for vaccination and exposure to thimerosal preservative has been discredited [17].

After screening, for category (a) eligible articles were included in the final results if they reported statistically significant associations between xenobiotic exposure and ASD risk. Prenatal to early postnatal (*i.e.* preconception to the second

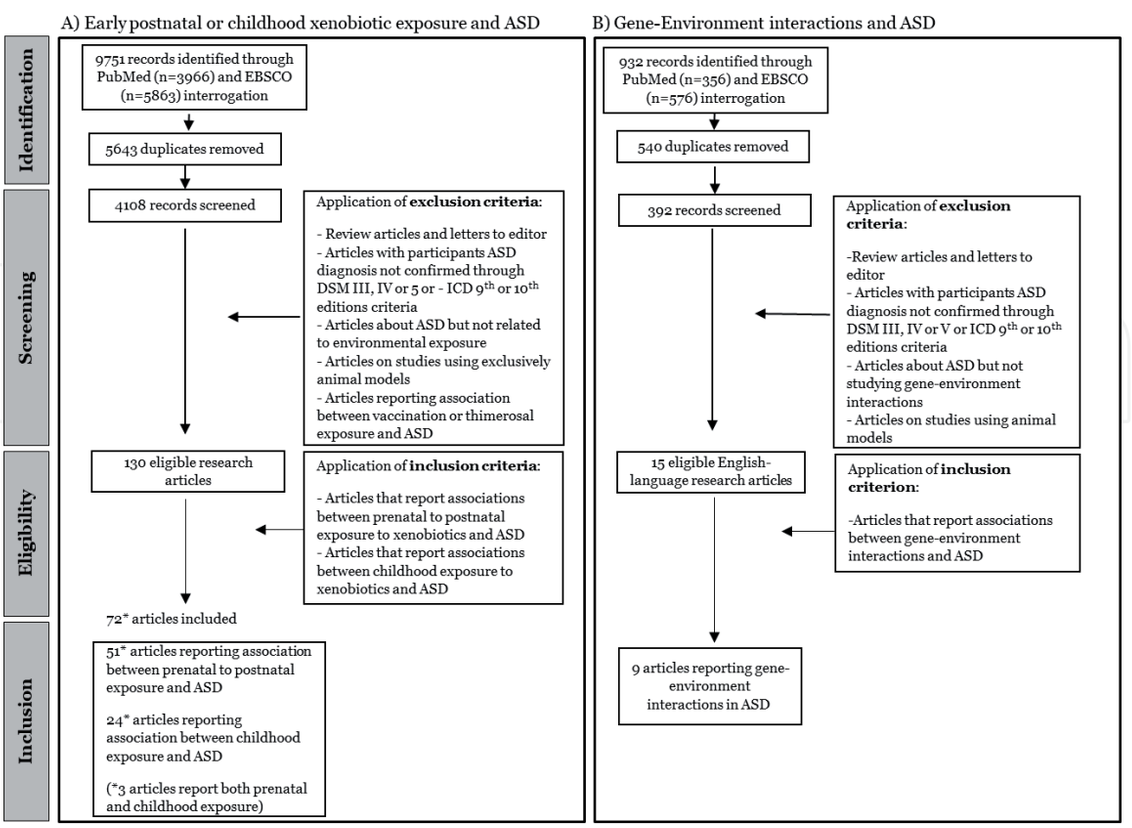

Figure 1.
PRISMA flowcharts for (1A) the identification of articles reporting associations between xenobiotic exposure and ASD; (1B) the identification of articles reporting associations between gene–environment interactions and ASD.

year of life) and later childhood exposure were considered separately. For category (b) eligible articles were included if they implicated gene–environment interactions in ASD risk, as long as the environmental component was the exposure to any of the xenobiotics' identified in category (a).

3. Results

3.1 Xenobiotic exposure associated with ASD

Figure 1A shows the flowchart for the identification of relevant publications. After removing duplicates, a total of 4108 unique records were screened using the defined exclusion criteria, resulting in 130 eligible research papers. Application of the inclusion criterion (*i.e.* to report an association between exposure and ASD) resulted in 72 articles selected to the final list of publications [18–89], shown in **Table S1**. From the 72 articles included, 51 (70.8%) reported prenatal to early postnatal exposure (up to 2 years of age), while 24 (33.3%) reported later childhood (from 2 years) to early adulthood exposure (**Table S1**). Three records reported association of both prenatal/early postnatal and childhood exposure with ASD [20, 28, 81].

The identified xenobiotics were categorized in seven major groups: Air Pollutants, Toxic Heavy Metals, Non-Persistent Organic Pollutants (non-POPs), Persistent Organic Pollutants (POPs), Pesticides, Pharmacological Drugs and Nutritional Factors (**Figure 2**). POPs include bisphenol A and phthalates, while non-POPs include polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and perfluorinated compounds (PFCs). The first five groups comprise ubiquitous toxins present in air, daily use products and the food chain, while exposure to the last two groups occurs through ingestion.

Historical proof-of-concept evidence for a role of xenobiotic exposure in ASD comes from three studies, which reported for the first time a very high prevalence of the disorder among subjects prenatally exposed to teratogens. Specifically, these studies reported an ASD prevalence of 4% among individuals exposed to thalidomide [63], of 8.8% in subjects exposed to valproic acid [59] and of 21.4% in individuals exposed to misoprostol [64] (**Table S1**).

Evidence supporting an association with ASD is stronger for exposure to air pollutants and pesticides, as all studies examining these toxins report an increased risk for the disorder (**Table S1**). Usually, these studies gather air quality or pesticide application data for large geographical areas and, by applying geocoding methods, investigate how exposure patterns relate to ASD prevalence. Each of these studies includes, at least, one hundred cases, with larger ones examining exposure in thousands of subjects [18, 22, 23, 29, 30, 52]. Environmental agencies are instrumental for collection of airborne pollutant and pesticide data in large populations from geographically defined areas, enabling valuable geocoding approaches. Because heavy metals can circulate in the air, large population geocoding studies, involving hundreds of subjects, are also applied to assess exposure to these chemicals [31, 32, 34]. Some studies quantifying exposure to heavy metals, as well as those that analyze POPs, non-POPs or vitamin D, need to resort to biological matrices. Because this data is so labor intensive to collect, most evidence comes from small datasets of less than one hundred subjects. For instance, this review identified 4 studies assessing heavy metals in biological matrices like hair, nails and teeth, all carried out in small numbers of subjects [33, 35, 36, 38]. Regarding POPs and non-POPs, evidence for an association with ASD is still limited, as fewer reports addressed these chemicals (**Table S1**). Two studies provide evidence for an increased risk of ASD from prenatal exposure to PCBs [43, 44] while, in other two, PFCs prenatal exposure was found to decrease ASD

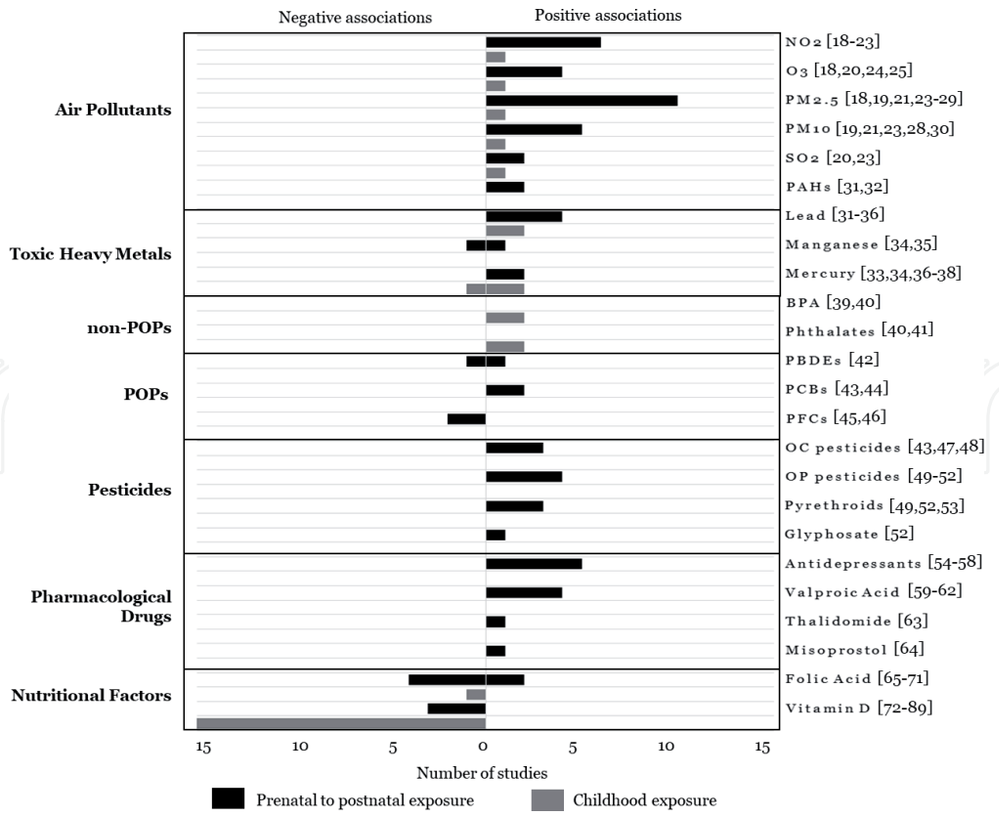

Figure 2.
Number of studies reporting negative and positive associations between exposure to xenobiotics and ASD, including prenatal to early postnatal and childhood to early adulthood exposures. NO_2 – Nitrogen dioxide; O_3 – Ozone; $PM_{2.5}$ – Particulate matter with a diameter less than 2.5 μm; PM_{10} – Particulate matter with a diameter between 2.5 and 10 μm; SO_2 – Sulfur dioxide; PAHs – Polycyclic aromatic hydrocarbons; BPA – Bisphenol a; PBDEs – Polybrominated diphenyl ethers; PCBs – Polychlorinated biphenyls; PFCs – Perfluorinated compounds; OC pesticides – Organochlorine pesticides; OP pesticides – Organophosphate pesticides.

risk [45, 46]. Concerning PBDEs, the only study reporting associations with the disorder observed a decreased risk due to exposure to BDE-153 and BDE-100 congeners, but an increased risk, only in girls, due to exposure to BDE-47 [42]. For bisphenol A and phthalates, two small size studies for each chemical report an increased risk of ASD associated with childhood exposure [39–41]. All studies on antidepressants report an increased risk of ASD (**Table S1**) and, as these usually resort to medical records to assess exposure, include thousands of subjects. A decreased risk of the pathology due to folic acid supplementation is observed by assessing medical records from large samples [68, 69]. However, two recent small size reports, which measured folic acid levels in maternal serum, show an increased risk of ASD associated with prenatal folic acid intake at very high concentrations [70, 71]. In case–control datasets a decreased risk for the disorder is associated with higher prenatal and childhood blood concentrations of 25-hydroxyvitamin D, the main circulating form of this nutrient, with mean serum concentrations values ranging from 9.9 ng/ml to 28.5 ng/ml in cases and 15.0 ng/ml to 40.1 ng/ml in controls [72–79, 83, 85–88]. Most of these studies comprise less than one hundred subjects, however 3 studies examining dried blood spots [84, 86] or medical records [81] were carried out in hundreds or thousands of subjects.

3.2 Gene-environment interactions associated with ASD

Figure 1B shows a flowchart for the identification of relevant publications. The query revealed 392 unique records, of which 15 remained after application of

exclusion criteria. Nine research articles reported gene–environment interactions in ASD (**Table 1**). The environmental component of these interactions included air pollutants (PM_{10}, NO_2 and O_3), PCBs, manganese and nutritional factors

Study	Genetic factor	Xenobiotic	N_{cases}|$N_{controls}$	Main conclusion
Schmidt et al 2012 [65]	677 C > T genotype in *MTHFR*	Folic acid	272|154	Daily prenatal maternal folic acid intake >600 μg was associated with a reduced ASD risk when the mother, the child or both had the low-activity 677 C > T variant.
Volk et al 2014 [21]	rs1858830 CC genotype in *MET*	NO_2 and PM_{10}	251|156	Carriers of the CC genotype with higher prenatal exposure to NO_2 or to PM_{10} were at increased risk of ASD when compared to subjects with CG or GG genotypes and lower exposure.
Rahbar et al 2015 [90]; and Rahbar et al 2018 [91]	Ile/Ile genotype of *GSTP1*	Manganese	100|100 [90]; 163|163 [91]	Among carriers of Ile/Ile *GSTP1* genotype, those with blood manganese concentrations >12 μg/L had higher risk of ASD.
Schmidt et al 2015 [92]	rs10741657 AA genotype in *CYP2R1*	Vitamin D	384|234	AA genotype associated with a decreased ASD risk when maternal vitamin D intake was <400 IU.
Coşkun et al 2016 [93]	rs2228570 TT genotype in *VDR*	Vitamin D	237|243	Trend for an association of the TT genotype with elevated circulating 25(OH) D levels in children with ASD.
Kim et al 2017 [94]	Copy number duplications burden	O_3	158|147	Higher burden of CNVs, namely duplications, and O_3 exposure increases ASD risk.
Mandic-Maravic et al 2019 [95]	*GSTM1*-null genotype	Any medication	113|114	Maternal use of medication during pregnancy associated with high ASD risk in offspring with a *GSTM1*-null genotype.
Bach et al. 2020 [96]	*GSTM1*-null genotype	PCB-153	169|169	Positive association between PCB-153 levels and ASD risk among carriers of *GSTM1*-null genotype, when adjusting for eating yogurt and fish, and paternal age at birth.

25(OH)D – 25-hydroxyvitamin D; CYP2R1 – cytochrome p450 2r1; GSTM1 – *glutathione S-transferase Mu 1; glutathione S-transferase Pi 1; IU – International Units;* MET – *MET proto-oncogene, receptor tyrosine kinase;* MTHFR – *methylenetetrahydrofolate reductase;* VDR – *vitamin D receptor.*

Table 1.
Gene–environment interactions reported in ASD. Listed are gene-environment interactions pairs associated with ASD as identified by the systematic literature review using PRISMA guidelines.

(folic acid and vitamin D), while the genetic component was a specific genotype or, in one study, the overall burden of copy number duplications (**Table 1**).

Most of the genes assessed in these studies are involved in the metabolism of xenobiotics. *GSTM1* and *GSTP1* encode glutathione S-transferases (GSTs), which catalyze the conjugation of substrates with reduced glutathione, easing their clearance from the organism. *VDR* encodes a nuclear receptor of vitamin D, whereas *CYP2R1* encodes a hydroxylase responsible for the conversion of this nutrient to its main circulating form (25(OH)D). *MTHFR* encodes a rate-limiting enzyme involved in folic acid metabolism. Contrary to the other genes, the *MET* gene is not directly involved in the metabolism of xenobiotics, but encodes a pleotropic tyrosine kinase involved in brain development through the MET signaling pathway [97].

4. Discussion

4.1 Exposure to xenobiotics and ASD

4.1.1 Air pollutants, heavy metals, POPs and non-POPs, and pesticides

Five attributes that are transversal to many of the xenobiotics reviewed in this study, including air pollutants, toxic heavy metals, POPs, non-POPs and pesticides, likely account for the increased risk of ASD associated with their exposure: 1) ubiquitous exposure; 2) bioaccumulation potential; 3) neurotoxicity; 4) endocrine-disrupting potential and 5) ability to cross physiological barriers.

Exposure to these toxins is ubiquitous, since they are present in the environment, in everyday household and industrial products, and in food. For airborne toxins, this ubiquity is exacerbated by transboundary flows of pollutants, a phenomenon in which toxins circulate long distances and deposit on land and water bodies far from their sources [98]. POPs exhibit high lipid solubility and low hydrophilicity, and are resistant to environmental degradation through chemical or biological processes, increasing their risk of bioaccumulation in human adipose tissue, the ecosystem and in the food chain [99]. Some air pollutants (*e.g.* PAHs), pesticides (*e.g.* OCs) and heavy metals (*e.g.* lead and organic mercury) are also persistent (resistant to degradation) and bioaccumulative chemicals [99]. For instance, methylmercury (MeHg), one of the main sources of organic mercury, can easily cross the blood–brain barrier and the placenta, and bioaccumulates in the brain, potentially leading to mercury poisoning [100]. Conversely, non-POPs like bisphenol A and phthalates are quickly excreted through feces and urine [101], but their presence in everyday products (*e.g.* water bottles and canned food, cosmetics and personal care products, and toys) and industrial activities is extremely widespread, rendering exposure to these chemicals continuous and universal.

Most of these toxins have well established neurotoxic properties [102]. Many, including bisphenol A, phthalates, pesticides, PAHs, PCBs, PBDEs and lead, are also endocrine-disrupting chemicals (EDCs), defined as any "*exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*" [103]. While EDCs were initially strictly defined as mimics of estrogens, androgens and thyroid hormones, acting as both agonists or antagonists to hormone receptors, it is now accepted that they act through much broader mechanisms [104]. EDCs interact with neurotransmitter receptors and transcriptional co-activators [104], and have been implicated in dysregulation of trafficking and signaling pathways [105], as well as of epigenetic mechanisms [106]. Early exposure to these toxins, which have overlapping neurotoxic and endocrine-disrupting properties, can therefore lead to

neurodevelopmental complications, with some coining the term neural-disrupting chemicals [107]. Experimental studies in humans and rodents have shown that most of these toxins cross both placental and blood–brain barriers [108, 109], enabling their neurodevelopmental toxicity. The endocrine effects of these toxins may also contribute to the male bias observed in ASD diagnoses. A recent systematic literature review of studies published from 1970 to 2016, concluded that many EDCs exhibit gender-specific effects, and that the male brain seems to be more vulnerable to neurotoxicity [110]. Corroborating this hypothesis, some of the studies identified in this review report gender-specific associations [20, 34, 42, 51].

Given the awareness regarding the hazardous health effects of exposure to these toxins, restrictive policies or bans on their use are often legislated. These include bans on the agricultural application of harmful pesticides [111], the widespread production of bisphenol A-free baby bottles [112] and regulations on PCBs, PBDEs and PFCs production [113]. However, such legislations are not always fully effective. For instance, despite bans, exposure to POPs is still ubiquitous because of their resistance to degradation [113]. Restrictions on bisphenol A use led to replacement by analogues (bisphenol F and bisphenol S) for which harmful effects are also reported [114], and are therefore regrettable substitutions. The transgenerational effects of these toxins are also important, as they can affect not only the exposed individual, but also subsequent generations, through epigenetic mechanisms [99, 104]. Most of the identified chemicals have been persistently used since the 1950s, leading to a growing environmental burden and accumulation of insults over several generations. Consequently, some authors speculate that these delayed effects may account in part for the steady prevalence increase in ASD reported in the last decades [115].

4.1.2 Pharmacological drugs

The increased prevalence of ASD among subjects prenatally exposed to three pharmaceutical drugs (thalidomide, valproic acid and misoprostol) provided the first strong evidence for the involvement of environmental risk factors in ASD. Thalidomide is an immunomodulatory drug, widely prescribed to alleviate morning sickness in pregnant women during the 50s, while misoprostol is a prostaglandin analogue used as an abortion inductor and valproic acid is prescribed for epilepsy and bipolar disorder. These drugs are teratogens (*i.e.* agents that alter the growth or structure of the developing embryo or fetus, causing birth defects), and likely induce brain damage leading to behavioral and cognitive deficits [116]. Nowadays, thalidomide is no longer used during gestation, while the intake of misoprostol and valproic acid by pregnant women is contraindicated.

We also identified 5 research articles associating maternal antidepressant intake during pregnancy with ASD risk, particularly for Selective Serotonin Reuptake Inhibitors (SSRIs). SSRIs act by increasing the extracellular levels of serotonin and are known to cross the placenta [117] and to be secreted through breast milk at low levels [118]. Increased serotonin levels have repeatedly been found in blood samples from ASD subjects [119]. While individual research studies report associations between prenatal exposure to antidepressants and ASD, a recent meta-analysis [120] underpins the inconsistency of overall findings. Thus, a clinical balance between the risks of untreated maternal depression and unclear neurodevelopmental risks of antidepressant exposure for the offspring is warranted.

4.1.3 Nutritional factors

The most encouraging results for protective factors for ASD come from studies examining disease risk and nutrient sufficiency. Overall, there is significant evidence

that folic acid and vitamin D supplementation during pregnancy and childhood are prophylactic for neurodevelopmental disorders.

Folic acid promotes the closure of the neural tube, reducing the risk of early neurodevelopmental problems: periconceptional folic acid intake prevents up to 70% of neural tube defects, with national health agencies recommending that women of childbearing age take 0.4 to 1 mg folic acid daily prior and during gestation [121]. However, while the natural folate is initially metabolized in the gut, folic acid is mainly metabolized in the liver, where the activity of dihydrofolate reductase (DHFR), the enzyme that converts folic acid to its biologically active form tetrahydrofolate, is reduced [122]. Thus, sustained high folic acid supplementation may eventually become noxious due to the accumulation of unmetabolized folic acid [122]. In agreement, two studies have observed a higher risk of ASD when mothers consume extremely high levels of this nutrient during pregnancy [70, 71].

Vitamin D plays a fundamental role in calcium and phosphorus metabolism, and is therefore crucial for various biological processes, among which the maintenance of brain homeostasis. Animal studies have also shown that the vitamin D receptor (VDR) is expressed in the brain since early in development [123]. Despite the growing number of studies reporting insufficiency of vitamin D in children with ASD, ambiguous cut-off levels for vitamin D insufficiency render difficult comparisons between studies [124].

4.2 Gene-environment interactions in ASD

The identification of consistent environmental risk factors for ASD is very relevant in view of the failure of genetics to fully explain the disease etiology and the clinical spectrum. However, integrating the emergent data on environmental risk factors for ASD with established genetic findings has been challenging. In this systematic review we identified 9 studies reporting specific gene–environment interaction pairs in ASD.

Because most of the identified genes cluster in biotransformation processes, their dysregulation may result in a deficient metabolism of xenobiotics, inducing pathological mechanisms that contribute to ASD onset. *GSTM1* and *GSTP1* are expressed in the brain, where they degrade multiple toxins [108]. Brain-expression of *MTHFR* and *VDR*, which are involved in metabolism of folic acid and vitamin D respectively, supports the importance of these nutrients for brain function. The mechanisms through which variants in the *MET* gene and air pollutants interact are unclear, but the role of the MET protein as a key signaling molecule during neurodevelopment [97] suggests that the genetic component of gene–environment interactions pairs goes beyond xenobiotics-responding proteins. In fact, *MET* is a known strong candidate gene for ASD [97], as are *GSTM1*, *MTHFR* and *VDR*, albeit with less supportive evidence.

While relatively scarce, the identified studies already offer valuable insights supporting the potential for preventive strategies based on environmental predictors for subjects carrying a genetic susceptibility variant. For example, controlling exposure to high levels of NO_2 or PM_{10} of carriers of the *MET* gene rs1858830 CC genotype could potentially lower their risk for ASD [21]. For subjects carrying a low-activity variant in *MTHFR* gene, which encodes for methylenetetrahydrofolate reductase, the risk of ASD might be mitigated by an adequate daily intake of folic acid during pregnancy [65]. In these 9 studies the environmental component of the gene–environment interaction pairs includes air pollutants (NO_2, PM_{10} and O_3), a PCB congener, manganese and nutrients (folic acid and vitamin D), opening many possibilities for prevention. While exposure to some factors (eg. outdoor air pollution) may be difficult to control, changes in nutrients intake are easier

to implement, and are particularly important for ASD-subjects carrying known specific variants in genes like *DHFR* [125], *MTHFR* [126] and *VDR* [127].

4.3 Strategies to assess early environmental exposure in ASD: the exposome

In 2005, Wild introduced the term "*exposome*" for a concept that complements the genome, and defined it as *"life-course exposures (including lifestyle factors), from the prenatal period onwards"* [128]. Unlike the genome, the exposome is highly dynamic, and is so diverse that no single technique will be able to completely quantify it. The large number of non-genetic risk factors associated with ASD reflects this, as such factors include not only environmental exposure to xenobiotics, but also psychosocial and lifestyle parameters. The 9 studies identified in this review tended to focus on a single or on a few environmental factors, employing the measuring method best suited for each factor. However, the simultaneous consideration of a set of exposures may much better describe the impact of the environment in individuals, and there are already a number of such studies under development for ASD.

To assess environmental exposure in ASD, the prospective cohort study MARBLES [129] recruits pregnant women who already have a biological child with the disorder, and are therefore at higher risk of a second child with ASD. The MARBLES study collects longitudinal information from the children, up to 36 months old, including environmental exposure, genetic and clinical data. This design allows the assessment of pre and early post-natal exposure to risk factors that may contribute to ASD risk. Because participants are recruited before or during pregnancy, monitoring of gestation and early childhood offers a chance to accurately measure exposures, allowing for the identification of early biomarkers.

Other studies with similar designs apply spectrometric methods to quantify the levels of toxins or their metabolites in biological matrices, usually through the collection of blood [42–45], urine [39, 41, 51] or hair [33, 38] samples. However, prospective designs are not always possible, and cross-sectional studies do not allow assessment of past exposures. Retrospective studies are a viable alternative, benefiting from new methods that allow assessment of previous exposure [14]. For instance, vanguard studies are now using naturally shed deciduous teeth [35] to retrospectively quantify exposure to xenobiotics in ASD subjects. During odontogenesis, deciduous teeth store signatures of exposure to chemicals, from the second trimester *in utero* until their replacement by permanent teeth [35]. The neonatal line, which is formed at birth, marks a histological feature that differentiates pre- and postnatally formed tooth layers. Consequently, teeth can be used to capture both the dose and timing of past exposures.

Another promising matrix takes advantage of archived dried blood spots collected through population-wide newborn-screenings for metabolic and congenital diseases. Chemicals relevant for ASD have been successfully detected in archived blood spots, including bisphenol A, PFCs, lead, mercury, PBDEs and PCBs [130, 131]. When correctly collected and stored, analytes remain stable in neonatal spots for years.

Other retrospective studies employ geo-referencing methods to collect information regarding exposure to air pollutants, pesticides and some heavy metals [18, 19, 24, 27, 32, 34, 49, 52]. These studies leverage indoor and outdoor air quality data, usage of agricultural pesticides or the location of environmentally-significant sites (*e.g.* landfill sites and high-intensity traffic roads) and apply geographic information systems techniques to infer potential associations with ASD risk. Early-life exposure questionnaires can also be used as a tool to assess past exposures and events [50, 66]. Finally, medical and prescription records and registries may be consulted when studying pharmaceutical drugs or supplement intake [54, 56].

Overall, a comprehensive analysis of the exposome must address a multiplicity of factors that includes not only exposure to chemicals in variable settings and situations, but also medical procedures, events and lifestyle, psychosocial and cultural variables.

4.4 Shift towards integrative strategies that address gene-environment interactions in ASD

All research studies identified in this review that report gene–environment interactions in ASD, published up to November 2020, examined specific xenobiotics (**Table 1**). Knowledge regarding interactions between genetics and the environment is vast outside of ASD context, and might be the basis to define what specific interactions to analyze. Leveraging from public, manually curated, literature-based resources, such as the *Comparative Toxicogenomics Database* [132] and the *Toxin and Toxin-Target Database* [133], that compile gene–environment interactions data, is fundamental. Genomic information, including SNV and CNV data, is also available from large international consortiums that aimed at detecting variants in ASD patients, such as the Autism Genome Project [4], the Simons Simplex Collection [134] and the Autism Sequencing Consortium [135]. For subjects for whom genetic data is already available or is currently being generated, an effort to collect exposure data might be very rewarding.

Given the emerging evidence highlighted by this literature review, there is a clear need to shift from studies that separately address the role of genetics and the environment towards multidisciplinary strategies that explore both components as interacting risk factors. Such strategies will inform about the mechanisms through which environmental exposure interacts with genetic background, contributing to ASD onset. Models must further consider ASD phenotypic and genetic heterogeneity. To fully understand the etiology of this very complex disorder, genetic, environmental exposure, epigenetic and clinical data needs to be collected simultaneously for the same group of individuals. It is possible that different gene–gene and gene–environment interactions are associated with distinct clinical subgroups of individuals with ASD and, consequently, phenotypic stratification may also be incorporated into study design. Conceiving such designs is challenging, especially given the large population datasets that are needed to achieve statistical power for the discovery of small-effect variables associated with the disorder [136, 137]. Artificial Intelligence (AI) methods, including data mining and machine learning algorithms, will be crucial to overcome the challenge of integrating substantial amounts of data, allowing the detection of environmental exposure patterns contributing to ASD onset.

4.5 Biological mechanisms underlying gene-environment interactions in ASD

Understanding the biological mechanisms underlying gene–environment interactions that contribute to ASD is fundamental to distinguish between causal and non-causal exposures identified through association studies. While knowledge on this is still limited, given the diversity of risk factors it is likely that multiple mechanisms converge in ASD etiology.

Genetic mutations rendering some individuals more susceptible to certain xenobiotics is the simplest gene–environment interaction mechanism. For instance, a gene functional polymorphism that inhibits the enzymatic degradation of a given toxin may lead to its detrimental accumulation in the organism. Many xenobiotic-responding enzymes, like cytochrome P450 enzymes and GSTs, are expressed in the brain, suggesting the occurrence of metabolic processes that inactivate toxins locally [108].

Epigenetics, a gene expression regulatory process that involves heritable and reversible biochemical modifications of DNA or histones, independent of the DNA sequence, acts at the interface between genes and the environment. These processes include DNA methylation, histone methylation and acetylation events, and post-transcriptional regulation by non-coding RNAs, which are known to be involved in brain development [138]. Environmental factors can modulate genetics through epigenetic mechanisms and xenobiotics implicated ASD are known to alter epigenetic patterns. For instance, valproic acid inhibits histone deacetylases up-regulating the expression of various genes [139]. 5-MethylTHF, a metabolite of folic acid produced by MTHFR enzymatic activity, is a donor of the carbon group used to methylate DNA [140]. Consequently, *MTHFR* gene polymorphisms that result in a diminished activity of the enzyme (i.e. *MTHFR* 677C > T polymorphism) might affect methyl donation and lead to impaired epigenetic regulation [141]. Epigenetic effects of air pollutants [142], BPA [143] and PCBs [144] have also been described.

Neuropathological mechanisms that putatively lead to ASD, such as oxidative stress, neuro-inflammation, hypoxic damage, abnormal signaling pathways and endocrine disruption, can be induced by exposure to xenobiotics. Reduced brain levels of glutathione, the major endogenous cellular antioxidant responsible for the detoxification of xenobiotics, and other oxidative stress biomarkers have been observed in ASD subjects [145]. Evidence for increased levels of neuroinflammation biomarkers in ASD, including brain levels of pro-inflammatory cytokines and microglia activation, which may be stimulated by allergens such as pesticides, has been reported [146]. Proxies for fetal and newborn hypoxia, indicating a deprivation of oxygen supply, have been reported in neonates that later develop ASD [26] and may be elicited by early-life events. Xenobiotics also interact directly with intracellular neurotransmitter pathways [108] leading to signaling impairments. For example, acetylcholinesterase, the enzyme that catalyzes the acetylcholine neurotransmitter breakdown, is the primary target of inhibition by organophosphate pesticides [147] Most of the identified xenobiotics are endocrine disruptors and a role for hormonal imbalances in the disorder is plausible, particularly given the male skewness in ASD diagnoses. Atypical steroidogenic activity, namely increased androgen [148] and estrogen [149] levels in the amniotic fluid, has been reported in affected males. Gender-specific effects of environmental toxins [110] and consequent hormonal imbalances may also be implicated in the female protective effect, a hypothesis proposed to explain the ASD male bias.

A novel area of interest in ASD is the role of gut-brain axis, which refers to biochemical signaling connections between the gastrointestinal tract and the central nervous system. Dysbiosis of the gut microbiome likely accounts for a high comorbidity of gastrointestinal symptoms in ASD patients [150]. While the liver is the predominant site of xenobiotic metabolism, the gastrointestinal tract is the first line of defense against ingested compounds, and is rich in both host and microbial enzymes. As the gut microbiota metabolize hundreds of dietary, pharmaceutical and industrial chemicals, dysbiosis could lead to impairments in the gut-brain axis resulting in neurological insults.

5. Conclusion

This review highlights the accumulating evidence for a role of exposure to xenobiotics in ASD risk, and reinforces the need of developing strategies that consider genetics and the environment as interacting components in ASD etiology. This is

further supported by the still limited but promising results originating from studies that explore gene–environment interactions.

However, the current knowledge is likely just the tip of the iceberg. Given the enormous progress in high throughput methodologies for analysis of biomolecules (genomics, transcriptomics, proteomics, metabolomics), together with the development of comprehensive surveys on environmental exposure and advances in artificial intelligence methods for the integrative analysis of large amounts of data, the field is ripe for new discoveries. The expectation is that knowledge of the exposome of individuals can be integrated with their genomes to define patterns of interactions that cause their particular configuration of behaviors in the autism spectrum. There are however many challenges ahead, particularly concerning the collection of such extensive information from patients in sufficient numbers for integrative analysis.

Because environmental exposure is amenable to adjustment or avoidance, the most important clinical outcome of better understanding gene–environment interactions in ASD is the potential for mitigating risk by controlling exposure of individuals with a genetic vulnerability. This line of research thus opens novel and important perspectives to future prevention and personalized interventions for ASD.

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Conflicts of interest

The authors declare no conflict of interest.

Appendix

Xenobiotic	Study	N_{cases}/ $N_{controls}$	Time of exposure	Exposure assessment	Risk
NO_2	Becerra et al. 2013	7421/ 72253	Prenatal	Geocoding/air quality	Increased
	Volk et al. 2013	279/ 245	Prenatal to postnatal	Geocoding/air quality	Increased
	Jung et al. 2013	342/ 48731	Prenatal to childhood	Geocoding/air quality	Increased
	Volk et al. 2014	251/156	Prenatal	Geocoding/air quality	Increased
	Raz et al. 2017	2098/ 54191	Postnatal (9 m)	Geocoding/air quality	Increased
	Ritz et al. 2018	15387/ 68139	Postnatal (9 m)	Geocoding/air quality	Increased

Xenobiotic	Study	N_{cases}/ $N_{controls}$	Time of exposure	Exposure assessment	Risk
O_3	Becerra et al. 2013	5839/ 55757	Prenatal	Geocoding/air quality	Increased
	Jung et al. 2013	342/ 48731	Prenatal to childhood	Geocoding/air quality	Increased
	Kaufman et al. 2019	428/ 6420	Postnatal	Geocoding/air quality	Increased
	McGuinn et al. 2020	674/855	Prenatal 3rd trimester	Geocoding/air quality	Increased
$PM_{2.5}$	Becerra et al. 2013	5839/ 55757	Prenatal	Geocoding/air quality	Increased
	Volk et al. 2013	279/245	Prenatal to postnatal	Geocoding/air quality	Increased
	Volk et al. 2014	251/156	Prenatal	Geocoding/air quality	Increased
	Raz et al. 2015	245/ 1522	Prenatal	Geocoding/air quality	Increased
	Talbott et al. 2015	217/226	Prenatal	Geocoding/air quality	Increased
	Chen et al. 2018	124/ 1240	Postnatal to childhood	Geocoding/air quality	Increased
	Ritz et al. 2018	15387/ 68139	Postnatal (9 m)	Geocoding/air quality	Increased
	Kaufman et al. 2019	428/ 6420	Prenatal to Postnatal	Geocoding/air quality	Increased
	Jo et al. 2019	2471/ 243949	Prenatal 1sttrimester	Geocoding/air quality	Increased
	McGuinn et al. 2020	674/855	Postnatal (1st year)	Geocoding/air quality	Increased
PM_{10}	Volk et al. 2013	279/245	Prenatal to postnatal	Geocoding/air quality	Increased
	Volk et al. 2014	251/156	Prenatal	Geocoding/air quality	Increased
	Kalkbrenner et al. 2015	979/ 14666	Prenatal 3rd trimester	Geocoding/air quality	Increased
	Chen et al. 2018	124/ 1240	Postnatal to childhood	Geocoding/air quality	Increased
	Ritz et al. 2018	15387/ 68139	Postnatal (9 m)	Geocoding/air quality	Increased
SO_2	Jung et al. 2013	342/ 48731	Prenatal to childhood	Geocoding/air quality	Increased
	Ritz et al. 2018	15387/ 68139	Postnatal (9 m)	Geocoding/air quality	Increased
PAHs	von Ehrenstein et al. 2014	104/ 53181	Prenatal	Geocoding/air quality	Increased
	Talbott et al. 2015 (2)	215/ 4856	Prenatal	Geocoding/air quality	Increased

Xenobiotic	Study	N_{cases}/ $N_{controls}$	Time of exposure	Exposure assessment	Risk
Lead	Priya and Geetha 2011	45/50	Childhood (4-12y)	Hair and nails	Increased
	Roberts et al. 2013	325/ 22101	Perinatal (at birth)	Geocoding/air quality	Increased
	von Ehrenstein et al. 2014	348/ 78373	Prenatal	Geocoding/air quality	Increased
	Talbott et al. 2015 (2)	215/ 4856	Prenatal	Geocoding/air quality	Increased
	Arora et al. 2017	22/54	Postnatal (15w)	Deciduous teeth	Increased
	El-Ansary et al. 2017	35/30	Childhood (3-12y)	Red blood cells	Increased
Manganese	Roberts et al. 2013	325/ 22101	Perinatal (at birth)	Geocoding/air quality	Increased
	Arora et al. 2017	22/54	Postnatal (15w)	Deciduous teeth	Decreased
Mercury	Windham et al. 2006	284/657	Perinatal (at birth)	Geocoding/air quality	Increased
	Obrenovich et al. 2011	26/39	Childhood (up to 6y)	Hair	Decreased
	Roberts et al. 2013	325/ 22101	Perinatal (at birth)	Geocoding/air quality	Increased
	Priya and Geetha 2011	45/50	Childhood (4-12y)	Hair and nailS	Increased
	El-Ansary et al. 2017	35/30	Childhood (3-12y)	Red blood cells	Increased
BPA	Stein et al. 2015	46/52	Childhood (10.1 ± 3.7y)	Urine	Increased
	Kardas et al. 2016	48/41	Childhood (7.5 ± 2.9y)	Serum	Increased
Phthalates	Testa et al. 2012	48/45	Childhood (11.0 ± 5y)	Urine	Increased
	Kardas et al. 2016	48/41	Childhood (7.5 ± 2.9y)	Serum	Increased
PBDEs	Lyall et al. 2017 (1)	545/418	Prenatal 2ndtrimester	Maternal serum	Increased Decreased
PCBs	Cheslack-Postava et al. 2013	75/75	Prenatal (early pregnancy)	Maternal serum	Increased
	Lyall et al. 2017 (2)	545/418	Prenatal (2^{nd} trimester)	Maternal serum	Increased
PFCs	Lyall et al. 2018	553/443	Prenatal 2^{nd}trimester	Maternal serum	Decreased
	Long et al. 2019	75/135	Prenatal	Amniotic fluid	Decreased
OC pesticides	Roberts et al. 2007	465/ 6975	Prenatal 1sttrimester	Geocoding/ pesticides data	Increased
	Cheslack-Postava et al. 2013	75/75	Prenatal 1^{st}trimester	Maternal serum	Increased
	Brown et al. 2018	778/778	Prenatal 1^{st} or 2nd trimesters	Maternal serum	Increased

Xenobiotic	**Study**	**N_{cases}/ $N_{controls}$**	**Time of exposure**	**Exposure assessment**	**Risk**
OP pesticides	Shelton et al. 2014	486/ 315	Prenatal	Geocoding/ pesticides data	Increased
	Schmidt et al. 2017	296/ 220	Prenatal	Survey	Increased
	Philippat et al. 2018	46/102	Prenatal	Maternal urine	Increased
	von Ehrenstein et al. 2019	2961/ 35370	Prenatal to postnatal	Geocoding/ pesticides data	Increased
Pyrethroids	Shelton et al. 2014	486/315	Prenatal	Geocoding/ pesticides data	Increased
	Hicks et al. 2017	159/298	Prenatal	Geocoding/ pesticides data	Increased
	von Ehrenstein et al. 2019	2961/ 35370	Prenatal to postnatal	Geocoding/ pesticides data	Increased
Glyphosate	von Ehrenstein et al. 2019	2961/ 35370	Prenatal to postnatal	Geocoding/ pesticides data	Increased
Antide pressants	Croen et al. 2011	298/ 1507	Prenatal	Medical records	Increased
	Rai et al. 2013	4429/ 43277	Prenatal	Medical records	Increased
	Gidaya et al. 2014	5215/ 52150	Prenatal	Medical records	Increased
	Harrington et al. 2015	421/ 464	Prenatal	interview and medical records	Increased
	Rai et al. 2017	5378/ 249232	Prenatal	Interview and medical records	Increased
Valproic Acid	Moore et al. 2000	52	Prenatal	Survey	Increased
	Bromley et al. 2008	10/622	Prenatal	Interview and medical records	Increased
	Bromley et al. 2013	12/509	Prenatal	Interview and medical records	Increased
	Christensen et al. 2013	5437/ 630178	Prenatal	Medical records	Increased
Thalidomide	Stromland et al. 1994	100	Prenatal 1sttrimester	Medical records	Increased
Misoprostol	Bandim et al. 2003	23	Prenatal 1sttrimester	Interview	Increased
Folic Acid	Schmidt et al. 2012	429/ 278	Prenatal (early pregnancy)	Interview	Decreased
	Surén et al. 2013	270/ 84906	Prenatal (early pregnancy)	Survey	Decreased
	Al-Farsi et al. 2013	40/40	Childhood (3-5y)	Serum	Decreased
	Nilsen et al. 2013	234/89602	Prenatal	Medical records	Decreased
	Levine et al. 2018	572/ 44728	Prenatal	Medical records	Decreased
	Raghavan et al. 2018	86/1171	Postnatal (2-3d)	Maternal plasma	Increased
	Egorova et al. 2020	100/100	Prenatal	Maternal serum	Increased

Xenobiotic	Study	N_{cases}/ $N_{controls}$	Time of exposure	Exposure assessment	Risk
Vitamin D	Meguid et al. 2010	70/42	Childhood (5.3 ± 2.8y)	Serum	Decreased
	Tostes et al. 2012	24/24	Childhood (7.4 ± 2.7y)	Serum	Decreased
	Mostafa and AL-Ayadhi 2012	50/30	Childhood (8.2 ± 2.4y)	Serum	Decreased
	Neumeyer et al. 2013	18/19	Childhood (10.6 ± 0.4y)	Serum	Decreased
	Gong et al. 2014	48/48	Childhood (3.7 ± 1.2y)	Serum	Decreased
	Bener et al. 2014	254/254	Childhood (5.5 ± 1.6y)	Serum	Decreased
	Kocovska et al. 2014	40/40	Early adulthood (18.9 ± 2.9y)	Serum	Decreased
	Fernell et al. 2015	58/58	Neonatal	Dried Blood Spots	Decreased
	Magnusson et al. 2016	9882/ 499757	Prenatal to childhood	Medical records	Decreased
	Bener et al. 2017	308/ 308	Childhood (5.4 ± 1.7y)	Serum	Decreased
	El-Ansary et al. 2018	28/27	Childhood (7.0 ± 2.3y)	Plasma	Decreased
	Guo et al. 2018	332/197	Childhood (4.9 ± 1.5y)	Serum	Decreased
	Wu et al. 2018	310/ 1240	Neonatal	Dried Blood Spots	Decreased
	Arastoo et al. 2019	31/31	Childhood	Serum	Decreased
	Lee et al. 2019	1399/ 1607	Neonatal	Dried blood spots	Decreased
	Alzghoul et al. 2019	83/106	Childhood	Serum	Decreased
	Sengenc et al. 2020	100/100	Childhood	Serum	Decreased
	Petruzzelli et al. 2020	54/36	Childhood	Serum	Decreased

BPA – bisphenol A; d – days old; m – months old; NO_2 – nitrogen dioxide; O_3 – ozone; OC pesticides – organochlorine pesticides; OP pesticides – organophosphate pesticides; PAHs – polycyclic aromatic hydrocarbons; PBDEs – polybrominated diphenyl ethers; PCBs – polychlorinated biphenyls; PFCs – perfluorinated compounds; $PM_{2.5}$ – particulate matter with a diameter less than 2.5 μm; PM_{10} – particulate matter with a diameter between 2.5 and 10 μm; SO_2 – sulfur dioxide; w – weeks old; y – years old.

Table S1.
Studies reporting xenobiotic exposure associated with ASD, identified through systematic literature review. For each study the numbers of ASD cases (N_{cases}) and controls ($N_{controls}$), the timing of exposure (specific time-points of prenatal, postnatal or childhood periods are shown when stated by the referenced authors), the exposure assessment method, and the direction of association are listed (increased or decreased risk by exposure).

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