

Autism is an Acquired Cellular Detoxification Deficiency Syndrome with Heterogeneous Genetic Predisposition

James Lyons-Weiler*

The Institute for Pure and Applied Knowledge, Pittsburgh, PA, USA

*Corresponding author: James Lyons-Weiler, The Institute for Pure and Applied Knowledge, Pittsburgh, USA, Tel: 4127288743; E-mail: jim@ipaknowledge.org

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Abstract

Neurodevelopmental disorders, including Autism Spectrum Disorders, have a complex biological and medical basis involving diverse genetic risk and myriad environmental exposures. Teasing apart the role of specific stressors is made challenging due to the large number of apparently contributing associations, gene X environment interactions and phenomimicry [1]. Historically, these conditions have been rare, making causality assessment at the population level infeasible. Only a few vaccines have been tested for association with autism, and it has been shown that improved diagnosis only explains a percentage of the increase in diagnosis. Now, the rates are so high in some countries that public school programs cannot handle the large numbers of special needs students, and professionals are quitting their jobs due to security concerns. Here, I review evidence of the pathophysiology of autism that reconciles the apparent paradox between the high degree of causal heterogeneity in environmental toxins, the absence of a common "autism gene," and the high degree of genetic concordance (heritability) of ASD and ASD-like traits. In brief, the sampling of environmental toxins, and thus the environmental toxin sampling liability for ASD varies among families involving different local exposures following injury to normal cellular endoplasmic detoxification and mitochondrial processes from toxic metals. The literature strongly supports that autism is most accurately seen as an acquired cellular detoxification deficiency syndrome with heterogeneous genetic predisposition that manifests pathophysiologic consequences of accumulated, run-away cellular toxicity. At a more general level, it is a form of a toxicant-induced loss of tolerance of toxins, and of chronic and sustained ER overload (ER hyper stress), contributing to neuronal and glial apoptosis via the unfolded-protein response (UPR). Inherited risk of impaired cellular detoxification and circulating metal retoxification in neurons and glial cells accompanied by chronic UPR is key. This model explains the aberrant protein disorder observed in ASD; the great diversity of genes that are found to have low but real contributions to ASD risk and the sensitivity of individuals with ASD to environmental toxins. The hindrance of detoxification and loss of cellular energetics leads to apoptosis, release of cytokines and chronic neuroinflammation and microglial activation, all observed hallmarks of ASD. Interference with the development of normal complex (redundant) synapses leads to a pathological variation in neuronal differentiation, axon and dendrite outgrowth, and synaptic protein expression. The most general outcomes are overall simplification of gross synaptic anatomy and, neurofunctionally, a loss of inhibitory feedback and aberrations in long-term connections between distant regions of the brain. Failed resolution of the ER stress response leads to re-distribution of neurotoxic metals, and the impaired neurocellular processes lead to subsequent accumulation of a variety of additional types of toxins with secondary, sometimes life-threatening comorbidities such as seizures, with overlapping (not mutually exclusive) causality. Reduction of exposure to toxins known to cause mitopathy (mercury) and endoplasmic reticulum dysfunction (mercury and aluminum) during pregnancy and during the early years of development will reduce the risk of ER overload and ER hyper stress, and of ASD diagnosis. This knowledge has immediate clinical translational relevance: post-vaccination symptoms should be heeded as a sign of susceptibility to toxins; Vitamin D can be increased to drive the healthy early phases of the UPR, and mutations in ASD genes encoding proteins with high intrinsic disorder may contraindicate the use of aluminum and mercury for carriers of risk alleles. Clinicians should be alert to a patient's Vitamin D receptor (VDR) mutational status prior to recommending increased doses. Approaches to improving overall brain health in autistics must be de-stigmatized and given high priority. Reduction of lifetime exposures of industrial and agricultural toxins will improve brain health for the entire human population. Purely genetic studies of ASD, and studies that do not include vaccination as an environmental exposure with potential liability and interactions with genes, are unethical.

To qualify as science, studies must test plausible hypotheses, and the absence of association from poorly designed, unethically executed, and underpowered and unsound whole-population association studies have been harmful distractions in the quest for understanding. Skilled pediatricians and ob/gyns will seek evidence of genetic predisposition to environmental susceptibility in the form of non-synonymous substitutions in brain proteins that require ER-folding, and they will provide informed cautions on exposures (from all sources) to environmental toxins to patients and parents of patients with signs of metal and chemical sensitivity.

To aid in this, a list of ASD environmental susceptibility protein-encoded genes is presented. A clinical Exome sequence test, followed by loss of function prediction analysis, would point to individuals most susceptible to vaccine metal-induced ER hyper stress leading to failed cellular detoxification.

Keywords: Autism spectrum disorders; Endoplasmic reticulum; ER-associated degradation; ER amino peptidase 1; EOR ER Overload Response; PKR double-stranded RNA-activated protein kinase; PKR-like endoplasmic reticulum kinase; Stress-associated endoplasmic reticulum protein 1; Sarcoplasmic reticulum; Toxicant induced loss of tolerance; Unfolded-protein response

Introduction

Autism spectrum disorders are diagnosed via dysfunctions in social interactions and communication skills, repetitive and stereotypic verbal and non-verbal behaviours, and restricted interests. These are the main core symptoms. The rates of autism spectrum diagnosis in the youth of highly vaccinating countries have skyrocketed from 1 in 100,000 to as high as 1 in 36 (US). Studies that objectively assess the issue of the increase have determined that diagnostic substitution is only responsible for a percentage of the increase and diagnostic substitution is a flawed explanation for the ongoing increase [2,3]. No studies exist that indicate that a majority of the increase in ASD is due to diagnostic substitution and the methods of diagnosis have not changed since the adoption of DSM-V, yet the rates continue to increase. Genetic studies have revealed that while inheritance is high within families (>90%), and large numbers of genes contribute in an additive manner [4], variation at different genes drive ASD risk in different families, each gene contributes a tiny amount to total liability, and, importantly, there is ample room for environmental liability, with estimates for environmental liability between 38 and 58% [5]. Other studies have in fact found evidence for specific gene x environment interactions.

Numerous biological dysfunctions are observed in processes associated with ASDs, including reactive limited production of glutathione, oxidative stress, mitochondrial dysfunction, decreased methylation, intestinal impaired permeability and dysbiosis, and innate and adaptive immune dysregulation. Thimerosal reduces the expression of methionine, critical for proper methylation [6]. This is especially relevant given CDC's recommendation of influenza vaccination during pregnancy, when some influenza vaccines still contain thimerosal. Many individuals with ASD exhibit signs of depressed methylation [7], consistent with homocysteinuria. Increased toxic metal burden is common, and endoplasmic reticulum stress is reported. Cortical structures show variation, and, depending on the region of the brain, long-range connections can be weaker, or stronger in people with ASDs compared to neurotypicals. A direct role of mitochondrial disorder (MD) in ASD overall is rare (between 1-5%); in "classical autism", while only 1-5% had mitochondrial disorders, 10-20% of individuals with MD have an ASD endophenotype, and the specific pathophysiological type of MD varies greatly among patients [8]. An as-yet unrecognized consequence of MD with causal relevance to ASD seems likely given the joint role of the ER, mitochondria and Golgi system in cellular detoxification.

Cellular Detoxification Deficiency Syndromes

Numerous previous authors have pointed to evidence of a role of environmental toxins in ASD. However, a coherent process model or theory that can reconcile the genetic heterogeneity with the failure to

detect a population-wide association between ASD and vaccines has not been produced. Individuals with autism certainly have altered neurological structures consistent with an altered neuroglial-mediated synaptic development program. How this is related to toxins in vaccines and to other toxins in the environment hinges on important findings at the cellular level - and specific risk (that is, risk of ASD diagnosis in any given individual) from environmental toxins must be interpreted in light of individual genetic susceptibility.

Various other cellular detoxification deficiency syndromes exist, and each one involves malfunction of a particular or general pathway via by which organs or cells in tissue get rid of endogenous and exogenous toxins. These include cerebral ischemia, Alzheimer's disease, multiple sclerosis, amyotrophic lateral sclerosis, familial encephalopathy with neuroserpin inclusion, and sleep apnea. The endoplasmic reticulum dysfunction seen in each of these conditions is reported to be of unknown origin, or in some cases, due to genetic mutation. Endoplasmic reticulum dysfunction begins with protein misfolding (aka protein disorder), caused either by genetics (difficult to fold) or via environmental damage to protein folding processes. The misfolding initiates a predictable, well-characterized evolutionarily conserved "unfolded protein response" [9]. The UPR is an adaptive cellular survival mechanism induced by the accumulation of misfolded proteins in the ER lumen by membrane proteins Ire1, ATF6 and PERK [10]. It also involves coordination of cell death and survival between the ER and the mitochondria [11].

In ASD, not only is UPR present, the specific mechanisms by which misfolding occurs are also known. Misfolding can occur due to mutations in intrinsically disordered proteins, which are less robust due to shape-changing mutations, and due to environmental factors that impact protein-folding proteins such as ERAP1 [12]. In ASD, glutamate receptors are over-activated, mitochondrial dysfunction is evident, and calcium homeostasis is impacted [13]. In Alzheimer's disease (AD), toxic assemblages of protein (amyloid precursor protein) form aggregates with aluminum and silica. Both AD and ASD involve amyloid precursor proteins, which are aberrant protein aggregates, which, when combined with aluminum, form amyloid and amyloid plaques [14,15]. Neurotoxicity of aluminum in Alzheimer's involves ER stress and mitochondrial dysfunction ER stress response leads to a shut-down of protein synthesis, increased expression of ER chaperones, and enhanced protein degradation. If the UPR response is prolonged, cell death can result [16].

To date, over 850 genes have been found that are "associated" with ASD. A huge number of protein-encoding genes contribute to ASD risk, with no general theory or model producing a specific mechanism explaining how so many functionally unrelated genes can interact with environmental toxins to produce ASD [17]. Mutations in ER-associated genes are known to be involved in some ASD cases. Crider et al. found aberrant expression of ER-stress associated genes IRE1, ATF6, PERK, as well as three others (ATF4, XBP1, and CHOP), in the middle frontal gyrus of ASD subjects post-mortem, compared to controls [18]. They also found a correlation between the expression of ER-stress gene expression and the severity of stereotyped ASD behaviors.

Valproic acid is used routinely in mouse models of ASD and is a non-controversial known cause of autism. Kawada et al. found that pregnant mice dams injected with valproic acid gave birth to offspring showing gene expression signatures consistent with ER stress and related the ER stress to the aberrant neuronal maturation and suppression of dendrite outgrowth [19]. Lipopolysaccharides used to create animal models of autism, leads to ER failure [20]. Palsamy et al. also found that valproic acid, which is known to cause autism, causes ER stress and problems with the UFR, along with demethylation of a key protein (Keap1) that leads to cataracts [21]. ER stress levels also leads to a decrease in the expression of proneural factors Hes1/5 and Pax6, preventing neural cell differentiation [20]. Nestin and beta-II tubulin expression levels were also reduced, and dendritic length shortened, consistent with the common observation of impaired inhibitory/excitatory ratios in synaptic architectures in individuals with ASDs. This strong evidence of ER-stress responses involved in ASD reveals a central causal role for toxins that impair ER normal functions.

Injury to Endoplasmic Detoxification

Endoplasmic reticulum stress proceeds in four general stages: the ER overload response (EOR), the unfolded protein response (UPR), shut-down of protein synthesis, and apoptosis [22]. Misfolding of proteins encoded by a single gene retained in the ER can lead to the UPR [23]. ER/mito/Golgi cellular detox is missing in patients with intestinal bowel disease, owing to impeded protein folding [24]. Misfolded proteins can fail to oligomerize and have been found built-up in the endoplasmic reticulum [e.g, 25]. Thus, individuals with mutations in a variety of genes may be born with a risk of impaired ER function due to misfolded proteins, causing further aberrant protein trimming, and slow cellular detoxification. Because the endoplasmic reticulum, along with the Golgi system, is also responsible for normal cellular detoxification, these de novo mutations may make individuals less able to tolerate neurotoxins including methyl mercury from dental amalgams from their mothers [26,27,6,28]. Ethyl mercury from thimerosal (in some flu vaccines) and aluminum, which is found in many vaccines on the CDC schedule as an adjuvant in the form of various aluminum salts, which also impairs mitochondrial function [29].

Dosing of aluminum in vaccines is not based on dose-escalation injection studies in mouse or rat pups. Safety studies cited in the formulation of levels approved for used in vaccines used dietary exposure levels in adult animals, but in the end, were based on adjuvanticity alone. Further, an error led to the use of an assumed safe tolerable limit of 1 mg/kg/day (all sources), up from 1 mg/kg/week. Important studies impacting views on the tolerable doses of aluminum were impacted as this error propagated. Flaws in the designs of studies that supported current aluminum amounts in vaccines are cause for grave concerns over widespread use of aluminum in vaccines in the current CDC schedule. Mold et al. [30] reported high levels of aluminum in the brains of autopsied patients with ASD.

The wisdom of the use of aluminum in vaccines is now in doubt due to many lines of evidence, including the findings that monocytes pick up aluminum and are signaled to the brain via TNF- α , and that aluminum is part of amyloid. Metals and proteins form complexes, such as copper/aluminum protein aggregates that are difficult to deal with at a cellular level. Exposures to environmental sources of metallic toxins also reduce the efficacy of vaccines [31].

Numerous studies have found association of ASD and other NDDs and psychological disorders with a bewilderingly large number of environmental toxins [32]. Studies have found increased rates correlation with a variety of exposures. The evidence for a role for a diversity of environmental toxins is overwhelming, and includes:

- thalidomide
- valproic acid
- mercury [33-41]
- air pollution [42-45]
- aluminum vaccine adjuvants [37,2]
- coproporphyrin [46]
- chlorpyrifos [47]
- glyphosate [48]
- phthalates [12,49]
- PBDEs [50,51]
- PCBs [51,52]
- electromagnetic frequencies [EMFs;53]
- industrial chemical (parental exposure) [54, 55]
- acetaminophen exposure after vaccination [56-60]

Numerous studies have revealed that metal toxicity is increased in individuals with ASDs (especially lead, tin, antimony and thallium) [19]. This reflects not only exposure, but a deficit in cellular detoxification.

Bowers and Erickson (2014) found reports of specific G x E interactions that include:

- organophosphates (PON1 gene)
- pregnancy-related stress (ADRB2 gene)
- traffic-related particulate matter (pollution) (MET gene)
- periconceptional maternal prenatal vitamin (MTHFR, CBS, and COMT genes).

More general analyses find that "ASD genes" tend to include genes that encode proteins that provide barriers against casual intake of toxins [61] and that individual chemical sensitivities can be expected from individual or familial genetic variants in specific genes. Brain inflammation is a hallmark of ASDs [62], with gliosis at the center of aberrant intercellular signaling. Aluminum impairs astrocytic glutamate uptake [9]. It causes a build-up of glial fibrillary acid protein (GFAP) near the cell nucleus, and destruction of the actin cytoskeleton [63]. Astrocytic failure due to metal toxicities leads to excess glutamate, with classical innate immune crisis signalling (IL-6, IL-17; [64]), stimulating chronic microglial activation leading to aberrant synaptic pruning [65]. The signaling includes cytokine release from cells undergoing cell death, either via apoptosis, which can be intrinsically mediated due to UPR and ER overload, or mediated via microglial action upon both injured and viable neurons and neural precursor cells [66, 67].

Animal studies show that injection of aluminum causes long-term neurological problems, motor deficits and social impairments [68-71]. Rates of autism were reported by some studies to not change after thimerosal was excluded from most childhood vaccines. Two studies

found ASD to be increased in patients receiving non-thimerosal containing vaccines, or other apparently “positive” effects of thimerosal, but neither study considered that the exposure of infants to injected aluminum hydroxide had increased dramatically nor the interpretation of increased risk of health issues from aluminum-containing vaccines. The number of doses of aluminum-containing vaccines increased concurrent with additional changes to the schedule. The continued increase in the rates of ASD diagnosis beyond that explained by diagnostic substitution points to aluminum as an important neurotoxin to consider as an initiator of ER harm [2,3].

Phenomimicry and G x E are Understudied, but Define Environmental Susceptibility Genes

In the full ER Hyperstress model, metal toxicity contributes to ER Stress, leading to apoptosis, microglial activation and cycling of metals in the brain (Figure 1). The question is: which proteins are likely to misfold, contributing to ER stress? Many genes encoding proteins directly involved in ER and Golgi function are implicated in ASD, and many other genes carry variation that causes them to fold or be trimmed incorrectly and build up in the ER lumen. Waste and toxins in the ER are normally removed via the Golgi apparatus via endosomes, lysosomes and secretory vesicles. Impairment of this process at any level can lead to intracellular protein, metal, and organopollutant cellular toxicity. Mutations in genes critical for Golgi function are known in ASD, including REEP3; C3ORF58; SLC35A3; neurobeachin; KIRREL3; VPS13B; and TRAPPC6B [72-78]. ER genes associated with ASD risk include RELN (via upregulation of PDIA1) [79] and neuroligins [80]. Numerous genes associated with ASD have mutations that lead to the accumulation in the ER. A specific mutation in neuroligin 3 (R451C) [23] activates the UPR, and a truncating mutation in neuroligin 1 leads to accumulation within the ER lumen [81]. Similarly, a missense mutation in neuroligin-4 induces ER stress [82].

The R558Q mutation in the G-protein-coupled receptor 37 (GPR37) gene causes a build-up in the ER lumen. PICK1 interacts with RAB39B to control ER-to-Golgi trafficking and mutations in the RAB39B gene are associated with intellectual disability comorbid with autism spectrum disorder and epilepsy [83]. Mutations in the gene GPR85 associated with ASD risk cause ER stress reactions via their accumulation in the ER [84]. An in-frame deletion of three amino acids in the NHE6 gene is linked to X-linked intellectual disability and autism and leads to accumulation of the misfolded protein [85]. Reith et al. (2011) found that the deletion of the protein tuberin lead to ER stress; tuberin is a gene implicated in tuberous sclerosis complex with epilepsy, developmental delay, and autism.

One of the more important findings is that variation associated with ASD in the CNTNAP2 not only shows build-up in the ER lumen, but it also shows that the mutant protein led to ATF6 activation, a key signal of the UPR (ERAD) [86]. Momoi et al. [87] found UPR due to mutations in the CNTNAP gene. Similarly, mutations in CADM1 associated with ASD also lead to ER stress via induction of CHOP [88,89]. The Golgi system is also critically important for synaptic formation and function, and thus some genes can have a dual effect on neuronal phenotype (e.g., NBEA) [90,91]. While the importance of aberrant membrane trafficking has been seen for some time in ASD and related conditions owing to the process of synaptic vesicle formation [92], the critical role of the impact of these mutations on intrinsic cellular detoxification pathways has not been sufficiently recognized. The finding of such mutations does not indemnify environmental stressors; in fact, these individuals may be more susceptible to further detoxification damage than others. Genes critical for ER and Golgi waste removal can be considered “ASD environmental susceptibility genes.”

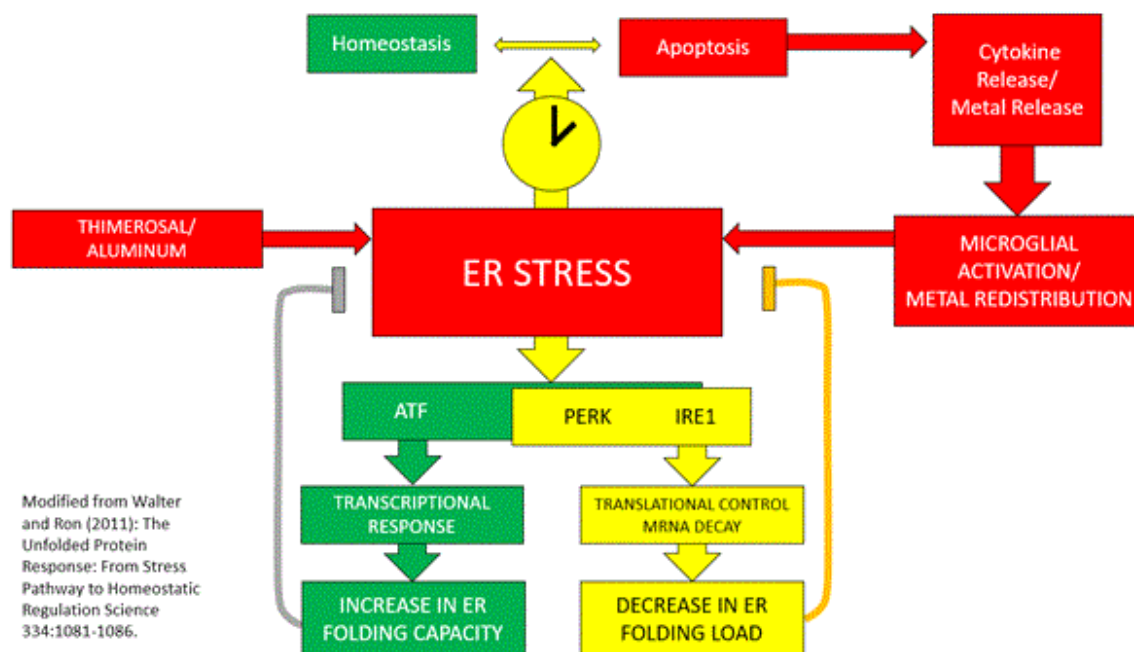


Figure 1: The canonical ER stress response pathway [93]. The canonical ER stress response pathway is activated in new cells due to the apoptotic release and redistribution of metals (and other toxins), spreading the ER response and initiating chronic microglial activation.

With astrocytic dysfunction, the excess glutamate contributes to chronic gliosis, which is both a consequence and contributor to aberrant pruning during development and throughout life. Any de novo or inherited mitochondrial disorder would tend to push the stress response toward apoptosis. The interplay between genetic risk and basic cellular function compromise realized via exposure to environmental toxins has implication for other neurological disorders. For example, Migdalska-Richards et al. (2016) [94] found that mutations in the GBA1 gene associated with a 20-30 fold increase in risk of Parkinson's disease (PD) elicited the UPR. Neurotoxic metals are central to the etiology of PD [95]. The link between Alzheimer's disease and aluminum is well-established [96].

Direct Vaccine Metal Intoxication and the UPR/ERAD/Apoptosis

The known specific mechanism by which metals induce ER stress include direct substrate binding, ROS and oxidative stress generation, and ER calcium release [97]. Mechanistic studies have found that aluminum impairs endoplasmic reticulum function [98]. Importantly, Stamogiannos et al. [99] found that thimerosal specifically inhibits the protein ERAP1, which, with ERAP2, trims peptides required for the generation of most HLA class I-binding peptides. This action of ERAP1 is essential to trim longer precursor peptides to correct length required for presentation on MHC class I molecules [100]. Bodewes et al. [101] found that annual vaccination against influenza reduces virus-specific CD8+T cell immunity in children. Recipients of the Vaxigrip vaccine (Sanofi Pasteur), which includes thimerosal, are more susceptible to upper respiratory infections involving viruses other than influenza [102]. Recipients of influenza vaccines are also less likely to have a successful defense against influenza from vaccination a year

later (Skowronski et al. 2017[103], and references 1-13 therein). The possibility that thimerosal directly impairs the adaptive immune system via ERAP1 dysregulation, leading to these immunological anomalies seems likely [99].

In neurodegenerative diseases such as Alzheimer's disease, ER-stress is at the core of low-grade chronic brain inflammation, a clinical hallmark of autism, and both astrocytic dysfunction and microglial activation result [104]. The adaptive UPR is seen as centrally important as well [105]. In ASD, chronic microglial activation can be expected due to the end-result of failure of UPR, ER overload and apoptosis due to a regular cycling of apoptosis, metal release, and cellular re-toxication. The evidence of gliosis in autism is very strong, and includes Vargas et al. [106], who found evidence of chronic microglial activation in post-mortem brains of people with autism from age 5-25. Blaylock reviewed [107] evidence of glutamatergic excitotoxicity in autism and Gulf War Syndrome and explored the roles of CMA in ASD. Pardo et al. [97] brought the neuroinflammation and gliosis observations together in ASD. The evidence is now even stronger [5,108]. See Edmonson et al. (2016) and Salter and Stevens (2017) for recent insights. None, however, have considered a role of ER stress and the unfolded protein response as the central link between environmental toxins and genetic variation [78,109,87,110,111,112].

Autoimmune factors (exposure to alloantigens that mimic human proteins) is known for some cases (e.g., congenital rubella infection; maternal and child anti-brain protein antibodies, which are now confirmed to cause structural cortical differences [113]. Autoimmunity to altered brain proteins may also result in recruitment of macrophages from the adaptive immune system in response to brain inflammation, which have been observed traveling to, and carrying aluminum directly to the periphery and into the brain [114,37]. Autoimmunity is

suspected in some cases of vitamin D deficiency associated with ASD [115].

The large number and diversity of weakly contributing genotypes with high familial inheritance has been partly recognized as contributing (via an as-yet unidentified process) to increased ASD risk due to genetic variation in a wide variety of proteins that have high protein intrinsic disorder (i.e. those that tend to not be stably folded in solution) [116]. This may involve as many as 1/3 of the proteins encoded by the human genome. This supports the theory that ER stresses make some individuals less tolerant of vaccine neurotoxins, and more susceptible to compounded effects of cumulative exposures of toxins. Inherited endoplasmic reticulum dysfunction risk would make individuals increasingly susceptible to impaired cellular detoxification, leading to accumulation of environmental toxins, protein toxicity, and a host of problems consistent with the findings of cellular and tissue pathologies observed in ASD [48]. De-activation of astrocytes reduces their ability to uptake glutamate- an excitatory amino acid-leading to apoptosis, release of cytokines, including IL-6, and microglial activation [110]. Mistiming of pruning by microglial

cells is problematic for both canonical development [65] and for availability of non-activated microglial cells for the establishment of sufficient complex synaptic architecture. Long-range connection to the thalamus are enhanced in ASD; [117] this may explain repetitive involuntary movements.

Mitopathy and ER Iatrogenicity of Vaccine Metals

Mitochondria drive all cellular processes of protein expression and secretion, as well as waste and toxin removal. Energy provision via ATP synthesis is only part of the key roles of mitochondria. Their importance in intracellular calcium flux via ER-mediated buffering, especially via the sarcoplasmic reticulum (SR), is also well established. Regulation of the localization and activity of mitochondria via the intracellular tubulin network are key for proper development signaling across synapses. Cellular pathologies involving mitochondrial transport and energetics have long been associated with neurodegenerative diseases; disruptions are recognized in autism as well. Proper ER functioning is also key for proper calcium flux [118].

Golgi Genes Associated w/ASD	Citation
REEP3	Castermans et al. (2007) [72]
C3ORF58	Dudkiewicz et al. (2013) [73]
SLC35A3	Edvarson et al. (2013) [74]
Neurobeachin	Niesmann et al. (2011);Nuytens et al. (2013);Volders et al. (2013) [77]
KIRREL3	Liu et al. (2015) [75]
VPS13B	Rejeb et al. (2017) [78]
TRAPPC6B	Marin-Valencia (2018) [76]
ER and UPR-Inducing Genes Associated w/ASD	Citation
RELN	Lammert et al. (2017) [79]
Neuroigin1	Tristan-Clavijo et al. (2015) [81]
Neuroigin2	Tu et al. (2017) [80]
Neuroigin3	Ulbrich et al. (2016) [23]
Neuroigin4	Zhang et al. (2009) [82]
GPR37	Tanabe et al. (2015) [119]
GPR85	Fujita-Jimbo (2015) [84]
RAB39B	Mignona et al. (2015) [83]
NHE6	Illie et al. (2014) [65]
Tuberin	Reith et al. (2011) [109]
CNTNAP	Momoi et al. (2009) [30]
CNTNAP2	Falivelli et al. (2012) [86]
CADM1	Fujita et al. (2010) [88]; Momoi et al. (2009) [30]

Table 1: ASD Environmental Susceptibility Genes Involved in or Impair Cellular Detoxification the Unfolded Protein Response [12].

Excess mercury was detectable in neonates when Hepatitis B vaccine included thimerosal [120,121]; the vaccine now contains 250

µg of aluminum, the safety of which has not been adequately studied for use in animals, premature infants and newborns. Aluminum is an

ROS-generator [122] and thimerosal is mitotoxic via the creation of de novo nicks and blunt-ended breaks in the mitochondrial genome [38].

Ethyl mercury (found in thimerosal) also induces the UPR in a manner that leads to cell death [123]. The combined mitotoxic and ER-stress effects of thimerosal and aluminum can no longer be ignored, and new dose-escalation studies with wild-type and mouse models focused on ASD genes related to UPR and those that require ER-folding are warranted [80].

Environmental Toxin Liability Sampling

Inherited risk of endoplasmic dysfunction would compound the toxicity of vaccines during pregnancy, perinatal and post-natal in a dose/weight dependent manner. One prediction of inherited cellular detoxification deficiency is the accumulation of other toxins unrelated to vaccines that reflect local environmental clines and concentrations. Impaired detoxification would lead to further neuroimmune effects of toxins. Boggess et al. [124] found a correlation between the mean serum levels of organic toxins and the severity of ASD behaviors in children with autism, but no such correlation was found in neurotypicals. Mean serum levels were also vastly higher in autistics compared to neurotypicals. The liability of environmental toxins, therefore, is additive and cumulative with - and interacts with- genetic risk in a manner consistent with the diffuse effects of variously misfolding proteins. Cytotoxic interactions between thimerosal and aluminum are highly likely. Ethyl mercury induces mutations in mitochondria in astrocytes, and aluminum impairs astrocytic cytoskeletal dynamics [38].

Strong evidence of microglial activation includes inflammatory cytokines and an excess of TNF- α in the CSF and serum of autistics [110,72]. TNF- α drives the UPR (Xue et al. [125]), and increased levels of TNF- α in autism [126] may indicate the presence of chronic and ongoing UPR. Nardone et al. (2014) [127] detected hypomethylation and upregulation of tumor necrosis factor-alpha (TNF- α) genes in the brains of individuals with ASDs [28].

Run-Away Toxicity Due to Toxicant Damage leads to ER Overload and Loss of Tolerance

The failure of the clearance of improperly folded proteins induced by aluminum and mercury deposited in brain cells can be expected to have a compounding, run-away effect in which cellular ER-mediated cellular detoxification becomes increasingly deteriorated by further accumulating toxins. Accumulated exposure will mean increased risk for certain individuals. This is consistent with the view of toxicant-induced loss of tolerance (TILT), suspected of playing a role in Gulf War Syndrome, Parkinson's disease, chronic fatigue syndrome, food allergies, including gluten sensitivity, fibromyalgia and multiple chemical sensitivity [128,129,130,131].

The added dimension of inherited risk due to risk of protein folding disorder is in the recognition that specific tissues express different proteins, and protein isoforms, and familial risk of ASD, at least for some families, will involve either function-altering mutations in proteins related to ER functions, including the UPR and EOR, or an increased propensity for brain proteins to misfold, adding to the risk of accumulation of misfolded proteins within the ER lumen. Cells with impaired ERAP1 will be vulnerable to expressivity of that risk, and will show increased vulnerability to oxidative injury, microtubular dysfunction, and reduced ability to secrete toxins out of the cells.

Why Past Association Studies Have Failed to Detect Association and Determine Causality

Numerous studies have addressed aspects of the theory that vaccines contribute to ASD, and the general view is that vaccines do not cause autism. Despite widespread claims to the contrary, some studies have found association [120]. A few troubling facts about the negative result studies exist that disallow the universal conclusion that vaccines do not cause autism:

Lack of Rigor: No randomized, saline-placebo-controlled prospective clinical trial has been conducted on the question of "vaccines" and autism. The majority of the studies conducted to date, reviewed below, only assess ecological correlation (association). Epidemiological studies such as these cannot determine (i.e., rule-in) causality; they can only determine whether a detectable correlation between exposure to a vaccine (or vaccines, depending on the study design) is found. If they cannot rule in causality, they also cannot rule it out, meaning the causal hypothesis is not adequately tested by ecological correlation studies.

"Vaccines" Not Tested: The second, equally important fact is that not all vaccines on the CDC paediatric schedule have been tested for association with autism. Most studies have focused exclusively on the MMR vaccine, and, in so doing, fall short of testing the hypothesis of an association between vaccines and autism.

No Genetic Subgroups Defined or Studied: None of the studies conducted on the question of association were designed to detect an association in a genetic subset of patients. By far, most of the studies conducted were not large enough to detect an association that exists due to risk isolated in a small (1-2%) of the population and were conducted at a time when far fewer vaccines were given to pediatric patients. The Wahlund effect is an artefact in population genetics in which an erroneous conclusion can be drawn due to masked (unknown or unmeasured) genetic heterogeneity in a population. Whole-population studies that ignore heterogeneity in genetic risk are likely to come to an erroneous conclusion about the association (or lack thereof) of an environmental factor with a phenotype. Unaccounted for, genetic heterogeneity can create either a false positive, or a false negative in a correlation study of environmental factors and phenotypes. Many rare alleles contribute to ASD risk (McClellan and King, 2010), meaning ample opportunity exists for missing specific genetic and environmental interactions in studies of environmental association only. The number of vaccines with aluminum jumped after 1986 when manufacturer liability for vaccine injury was removed by the National Childhood Vaccine Injury Act (NCVIA) of 1986. DeLong (2018) found that vaccines that were licensed after legislation that has preempted product liability lawsuits are associated with a significantly higher incidence of adverse events than were vaccines that were licensed during the period when consumers were permitted to sue. The specific genetic liability may have increased in the population as the vaccine schedule was expanded.

Causality is Not Tested by Ecological Studies: It is tautological that a study that is not capable of testing a hypothesis of causality will fail to provide sufficient weight of evidence in support of causality. Since prospective blinded randomized clinical trials comparing total health outcome of vaccinated vs. unvaccinated children have not been conducted, we are left with a slew of retrospective studies that only obliquely address the issue of causality, if at all; none these studies can disprove the role of mutations in ER-folded proteins as contributing to

vaccine-induced encephalopathy-mediated autism. Of the studies conducted often-cited as “disproving” that vaccines do not cause autism, seven merely analyzed trends, these include Dales et al. 2001; Kaye et al. 2001; Madsen et al. 2003; Stehr-Green, 2003; Honda et al. 2005; Fombonne et al. (2006); McMahon et al. (2008) [132-137] failing to provide a sufficient test of the hypothesis of causality. Kaye et al. [135] studied only MMR, and their study used no separate control group. Many of these studied the effect of only one vaccine (MMR only: Dales et al. 2001; Kaye et al. 2001; Madsen et al. 2003; Makela et al. 2004; Honda et al. 2005; Fombonne et al. 2006; MMR + Varicella vs. MMRV only: Klein et al. 2012; DPT only: Andrews et al. 2004; measles only: Baird et al, 2008; influenza vaccine only: Zerbo et al, 2017) or just the MMR and measles vaccine (Lingham et al. 2003). These studies cannot be used to assert that “vaccines” do not cause autism. Indeed, vaccines exist in the CDC pediatric schedule that have never been studied for association w/ASD. Zerbo et al. [138] suffered numerous additional flaws, including over-correcting for multiple hypothesis testing using the Bonferroni adjustment, which is generally considered too strict. Zerbo et al. along with all studies that considered only one vaccine are subject to confounding due to healthy user bias: patients who had adverse events prior to receipt of the MMR (such as febrile seizures or encephalopathy) without formal ASD diagnosis may have abandoned vaccination due to the adverse events. For a confirmed example in which healthy user or healthy family bias affected measurement of vaccine injury in a study see Glickman et al. (2017) [139].

Increased Risk of Aluminum Containing Vaccines Interpreted as Thimerosal Safety: Studies that focused on the relative risks of thimerosal-containing vaccines (TCV's) found either no differences in health risks compared to non-TCVs, or apparent benefits of TCVs over non-TCVs. These benefits could, and perhaps should have been interpreted as increased risk of non-TCVs, many of which, of course, contain aluminum (i.e., that aluminum containing vaccines were riskier than TCVs). The interpretations of “equally safe” at the time was epistemologically equivalent to “equally risky,” and therefore studies of TCVs vs. non-TCVs that reported apparent benefit should have alerted on increased risk due to non-TCVs, which of course contain aluminum (e.g. HepB vaccine was switched from TCV to aluminum around the time of these studies). The widespread design of studies that compared risks of categories of vaccines instead of comparing to true saline placebos have contributed to society's inability to measure actual risk. McMahon (2008) is included in a list of studies negating the ASD/vaccine link by the US Centers for Disease Control (CDC), but never addresses autism. It is, instead, a study of data from the Vaccine Adverse Events Reporting System (VAERS) that reported no difference in preservative and preservative-free vaccines with respect to injection site reaction, rash, or infections. At least three studies of TCV's found increased risk of tics and increased risk of language delay, a finding confirmed in 2015[35].

Off-Target Aims: Numerous other studies often cited as disproving a link between ASD and vaccines in fact did not address risk of ASD diagnosis from vaccines at all, and instead measured co-morbid conditions that are not part of the formal diagnosis of ASDs ; these include (Peltola et al. 1998; Davis et al. 2001; Black et al. 2002; Chen et al. 2004; D'Souza et al. 2006; Hornig et al. 2008; Klein et al. 2012; Destefano et al. 2013) [140-146]. Titles and abstracts from such studies sometimes include misleading statements (e.g., Peltola et al. analyzed no data on autism, yet the study is entitled “No evidence for measles, mumps, and rubella vaccine-associated inflammatory bowel disease or

autism”). Of course, no analysis means no evidence, but no evidence does not mean refutation.

Repeated Analysis of the Same Data, No Interaction Terms Studied: Three studies (Price et al. 2010 a, DeStefano et al. 2013, and Price et al. 2010 b) used the same data set and analysed the correlation of ASD rates with different variables derived from the same patients - a practice not recommended due to Type 1 inflation risk and due to a lack of ability to study interactions among variables. In fact, none of the epidemiological studies published interaction terms between covariates and vaccines; instead the default has been to report no of association with vaccines after “correcting for” these variables, which in many cases likely point to collinear risk factors, such as mother's income, gestational age, and body weight. Measures to avoid model overfit or to perform objective model selection are not reported in these studies [147].

Lack of Statistical Power: Most of the studies often cited are also likely too small to have had sufficient power to detect a positive association between the subset of vaccines studied and ASD considering the prevalence of ASD in the populations studied, which for most studies is between 1-2%, including most of those cited with other flaws thus far, and including Lingham et al. (N1+N2=567); Thompson et al. (N1+N2=1047); Tozzi et al. (N1=697; N2=706); Price et al. (N1=256; N2=752); Klein et al. (N1=77; N2=1540); Taylor et al. (N1+N2=473); Uno et al. (N1=189; N2=224) [148-154].

Three large studies (Heron et al. 2004; N>14,000; Makela et al. (N=535,544) [150]; Klein et al (N=715,484) [155] had design and focus issues similar to the smaller studies. Klein et al. (2012) only studied MMR+MMRV with or without varicella vaccine exposure, not “vaccines”, and their outcome measure was seizures, not ASD. The study is not relevant to the question of ASD risk from vaccines at all. Heron et al. (2004), like other studies that only focused on TCV vs. non-TCVs, may have in fact found aluminum-containing vaccines to be riskier than TCVs. They also “corrected for” birth weight, gestation, gender, maternal education, parity, housing tenure, maternal smoking, breastfeeding, and ethnic origins- without testing for interactions of these covariates with vaccination uptake. All three of these studies were retrospective ecological studies, only capable of assessing correlation, not testing causality, and none addressed the issue of ASD risk in an identifiable, albeit heterogeneous, susceptibility group. Makela et al. only studied MMR, and, like many other such studies, is a likely candidate for healthy user bias intrusion.

Use of Subjects as Their Own Controls: Self-controlled case series studies (SCCSs) have been used to assess trends and are cited as “controlled studies” in commentaries indicating lack of association [156]. While such designs impart higher statistical power, they do not protect from temporal confounding with overall trends, and often, as have other studies, focus on outcomes other than ASD diagnosis. Ward et al. [157] focused on hospitalizations from ASD and found an increased risk of febrile convulsions lasting >30 minutes six to 11 days after receipt of the MMR vaccine. In reality, most cases of ASD do not lead to hospitalization, and this study likely suffered from selection bias with respect to any information it provides on ASD risk. Temporal confounding is controlled in blinded, prospective randomized placebo-controlled clinical trials, but not in SCCS studies such as Taylor et al. [147] and Farrington et al. [158].

Animal Studies are at Odds with the Correlation Studies: When drugs are approved by the FDA, rigorous dosage safety testing of all components is required. Under US law, only the safety of the protein

portion of vaccines and other biologics is required. Numerous studies (too numerous to cite here, see Lyons-Weiler, 2016) give cause for grave concern over the injection of thimerosal [159,99] and aluminum into mammals with developing brains. Aluminum is widely recognized by most of the scientific community outside of vaccines as a serious neurotoxin. In addition to causing ER stress, aluminum also disrupts cytoskeletal dynamics and is an intracellular ROS generator that promotes neurologic disease. Mice injected with aluminum adjuvant doses equivalent to those given to US military service personnel showed both neuroinflammation and cell loss in the spinal cord and motor cortex, with consequent memory deficits (2007).

Error Propagation: These studies are among those cited by the American Academy of Pediatrics in 2013 (AAP, 2013), and again in 2017 (AAP, 2017), by the CDC (2018), and others (e.g., Hotez, 2017) who represent them as strong evidence of no link between “vaccines” and autism, when in reality the ecological studies conducted are not capable of ruling in, or ruling out vaccines as a causal factor and therefore do not represent a critical test of the hypothesis. When the literature cited in public health policy statements and statements designed to influence public health policies cite studies with no regard for the quality of level of evidence provided by studies they cite, accompanied by calls for less science on the topic, an anarchy of regulation of public safety will reign. Seven of these oft-cited studies are so small that less than one human subject would be expected to be found to have ASD diagnosis in either one or both study groups assuming prevalence on the order of 1-2% (i.e., Hornig et al. 2008; Fombonne et al. 2001; Black, 2002; D’Souza et al. 2006; Pichichero et al. 2008; Hornig et al. 2008; Klein et al. 2011). According to Google Scholar, these seven studies alone have been cited by over 1,000 other peer-reviewed, or editor-reviewed articles in professional journals. Hooker et al. [160] reviews serious flaws in these and other studies. Formal post-hoc power calculations for each of the studies reviewed here are underway. The Taylor et al. [161] meta-analysis, by definition, is seriously flawed for citing underpowered studies and studies subject to biases and limitations outlined.

Past Studies Were Not Designed to Consider, and Therefore are Irrelevant to, Genetic Susceptibility in a Minority of Patients

The UPR Response/ER hyperstress theory, or any other mechanistic theory that incorporates specific genetic susceptibility, is not in any way inconsistent with negative findings in whole-population ecological correlational studies. If a genetic susceptibility exists in a minority of patients, none of the past studies cited as disproving the hypothesis that vaccines contribute to autism risk are relevant. In the original Wakefield study, all patients were self-selected; all had both autism and gastrointestinal disorders, and this self-selection may have been the impetus for Dr. Wakefield and his co-authors to posit the hypothesis that vaccines may cause autism and called for more research on that question.

Many of the studies used to bolster the idea that vaccines do not cause autism (universally) are so flawed and internally inconsistent due to non-sequitur over-arching conclusions that they should no longer be cited in the scientific literature, nor in policy documents as showing that “vaccines do not cause autism.” Despite its volume, this body of science is very weak. CDC, AAP and others should no longer misrepresent the value of these studies to public policy. A common non-sequitur response to finding that vaccines may harm some individuals is that “vaccines save lives”; however, this does not address the scale or scope of the risk to a minority. If such a minority exists who cannot tolerate vaccines as well as a majority, and the majority

benefits from vaccines, it is the duty and moral obligation of the majority, who benefit from vaccines at the cost of life-altering injuries and deaths in the minority to protect them from further and future harm. Biomarkers studies are urgently needed to identify those with high specific (individual) risk.

Clinical Translational Significance and Consequences for Whole-Population Brain Health

Genetic predisposition to detoxification disability may be heterogeneous, but that does not preclude discovery of multiplex sets of genetic markers useful to predict risk. Public health policies that do not disenfranchise the genetic minority at risk are needed, and pediatric practices should be keen to adopt vaccine safety screening as standard practice, using genetic and biochemical tests developed using prospectively collected tissues for indications of risk post-injury. A list of brain proteins that predispose to ER stress-mediated thimerosal and aluminum sensitivity suitable for multiplex testing is needed; Table 1 only reflects those genes chosen to date by investigators out of interest in the study of ER stress in autism genes. The number of genes could be very large if mutation-driven variation alternative splicing due to mutations in promoters and introns also leads to protein folding challenges.

Neuroprotection and Reversal of Vaccine-Induce Cytotoxicity

Clearly, proper cellular detoxification requires healthy mitochondrial, Golgi and endoplasmic functions. Numerous approaches have shown promise in ASD in general, and their mechanisms of protection, too, shed light on fundamental cellular detoxification pathophysiology.

Mitochondrial Support and Ketogenic Diet

Numerous studies and reviews of studies of treatments of autism include reviews of enhancing mitochondrial activity with supplements and drugs. In short, variation exists within ASD on the efficacy of a variety of treatments, and clinical information is lacking for some treatments. Delhey et al. [162] studied variation in outcomes of patients with ASD using antioxidants, B12, B vitamins, multivitamin, CoQ10, carnitine, other vitamins or herbal supplementation and folate supplementation and found that nutritional and biological logic of enhancing mitochondrial support depends heavily on whether and which specific mitochondrial pathway was impacted. Cheng et al. [163] reviewed the knowledge base of high-fat, low-carbohydrate ketogenic diets (KD) on ASD in animals and humans and concluded that clinical studies are needed both to link KD to improved mitochondrial function and ASD phenotypes. Frye and Rossignol provide further insights, and Frye et al. [164] found improvement in language in ASD patients given folinic acid. Melatonin appears to afford protection against neurological changes associated with exposure to aluminum. These findings refute claims that autism “is genetic,” as do the genetic studies themselves, which provide estimates for liability of environmental factors for ASD risk as high as 50%.

Genetic Screening for Early Warning

Knowledge that aluminum and mercury can exacerbate genetic limitations on cellular detoxification means that the search for those most susceptible to vaccine injury can, in part, begin with a focus on mutations likely to impair protein folding in the ER. Reducing in utero and perinatal exposures to mercury and aluminum for individuals

with mutations that cause difficulties in protein folding should reduce the risk of run-away toxicity and improve brain development. Perinatal genetic testing paired with functional predictions of non-synonymous substitutions in intrinsically disordered proteins will point to individuals for whom vaccination is likely to contribute to endoplasmic reticulum stress [116].

Reduction of dietary sources of aluminum, lead and fluoride via filtration or replacement with silica-rich waters should be studied as a way to prevent total aluminum exposure. A study of silicic acid-rich mineral waters found reduction of total aluminum body burden and increase in cognitive performance in some study participants. Other participants showed no change, and some showed continued decline. Silicic acid drops are readily available for addition to food and water to adsorb and trap aluminum, preventing dietary absorption.

Additional clinical steps that can be explored post-vaccination include removal of beta-amyloid precursor protein (β APP) via intranasal insulin. β APP is found to be overexpressed in ASD w/ aggression. AD progression, Al-induced behavioral deficits and neurofibrillary tangles are reduced by deferoxamine (DFO) chelation.

Chelation of brain aluminum and iron may also be possible using intranasal deferoxamine Hanson et al. and Fine et al. [165]. Given the general failure of cellular detoxification seen in ASD due to ER overload, the stigma on metal detoxification by standard blood chelation must be lifted. Intranasal delivery of both insulin and DFO in ASD have the benefits of targeted administration, treatment cessation given side effects, and lower dosages, and both could prove worthwhile. Endogenous 6-hydroxydopamine (6-OHDA) is toxic to mitochondrial complexes I and IV, and intranasal DFO prevents 6-OHDA mitotoxicity. DFO treatment, given prior to injection of lipopolysaccharide, prevented microglial activation, TNF- α increase, and ameliorated deficits in cognitive performance [166]. Percy et al. [167] lamented the lack of progress in the use of low-dose intramuscular DFO as treatment for AD given a trial conducted in the 1990's showed that DFO provided a two-fold reduction of rate of progression of AD. It is an important read for anyone interested in options for reducing aluminum-induced neurotoxicity.

Both intranasal insulin and hyperbaric oxygen therapy (HBOT) stimulate de novo neurogenesis [168,169], thus aluminum/mercury brain burden amelioration could be preceded- and followed - by intranasal insulin to break up amyloid precursor proteins and stimulate neurogenesis. The DFO would capture the aluminum, and any iron, reducing ROS-mediated inflammation. Studies of such a protocol including HBOT to stimulate neuronal stem cells could provide a boon for overall brain health. Intranasal autologous plasma with stem cells is currently being studied for its effects on patients with various neurological dysfunctions [170]. Combined modalities that ignore aluminum brain detoxification can be expected to be of limited efficacy.

Reversing Widespread Hypovitaminosis D

Eyles et al. [171,172] found evidence that deficiencies in Vitamin D are associated with abnormal brain development and neuropsychiatric disease. Increasing evidence points to Vitamin D deficiency in ASD. Low gestational and early childhood levels of Vitamin D3 can be expected to have negative consequences on brain development in the face of genetic and environmental factors that initiate the UPR. Indeed, low levels are associated with autism [115,171-174]. Vitamin D seems protective against ER stress, as it induces the "healthy" aspects of the UPR response, clearing the ER of problematic proteins via early

response of BIP expression and XPB-1 splicing [175]. Increased intramuscular and oral vitamin D improves ASD symptoms [176,112], but among-study variation can be expected due to genetic heterogeneity. Further, complex disease phenotypes are the result of combined effects of genes and environment, and, such phenotypes such as intellectual disability may be reversible - but ignorance that the symptoms are due in part to neotoxins, no such treatments might be viable. Many inborn errors of metabolism are, in fact, treatable, or at least the symptoms ameliorated by simple vitamin supplementation [177]. Further, hypovitaminosis D is widespread; Papadimitriou (2017) reviews an error in the estimation of the dosage needed to achieve proper serum levels of vitamin D and reported that 8900 IU/d would be necessary to achieve useful levels (lower levels scaled for infants and children).

Vitamin D deficiency is not new, but is, instead, a precursor factor that exacerbates risk of neuroimmune injury from vaccine metals. Dr. Keith Baggerly (MD Anderson Cancer Center) has identified four key errors in the IOM's recommendations for Vitamin D serum target levels (Institute of Medicine. 2011). Specifically, the IOM had used the wrong denominator in calculating the risk of bone evidence in the cadavers of elderly patients; they did not have sufficient data to conclude that serum levels >4,000 IU had risk of toxicity; they used data reflecting bone health, ignoring the Vitamin D level requirements of various other tissues, and their estimate of the dose required to reach their recommended serum level in 97.5 % of population was inaccurate. Baggerly's re-analysis (Baggerly, 2017a; 2017b) leads to the conclusion that 3,000 IU are needed.

Cannell (2008) realized an important potential role for Vitamin D deficiency in ASD, and Grant and Cannell (2013) found an ecological correlation between UV-B level exposures across latitude and autism levels, suggesting that low Vitamin D from sunlight might increase ASD risk.

Bittker (2014), however, found exceptions for Vitamin D as a risk factor in autism, and indeed found evidence that very high levels of Vitamin D intake seem to correlate with ASD risk. Both positions are consistent with the ER hyperstress model because not all children's ER hyperstress induced by adjuvants will respond to Vitamin D alone. There are multiple risk factors in the ER hyperstress model and the details of the UPR may vary among lineages with different genetic heritages. Vitamin receptors may not be well expressed, and thus serum levels may not be reliable indicators of requirements at the cellular level. The original daily recommended dose (400-600 IU/d) should be updated for individuals with normal - and properly expressed and folded - Vitamin D receptors. Guidance from a medical professional should be sought for a reasonable increase.

Guo et al. [178] found that "appropriate" levels of Vitamin D increased hippocampal health in the context of diabetic neuropathy, so it seems likely that "more Vitamin D is better" may not apply in the case of chronic sources of ER stress. Mostafa and Al-Ayadhi [115] found that serum Anti-MAG is negatively correlated to serum Vitamin D levels; however, 1/3 of the proteins in the body require folding in the ER, and improperly resolved ER hyperstress will reduce the expression of any of those proteins, including those without a mutation. An immense number of proteins should be found to be reduced with chronic systemic ER stress. Still, immunomodulatory effects of vitamin D exist, including modulation of T-helper cell function and induction of CD4 (+) CD25 (high) regulatory T-cells [115]. The TH1/TH2 skew in ASD may also be explained via effects of ER hyperstress of vitamin uptake and processing.

Two open-label trials found that Vitamin D supplementation improved ASD symptoms between 75-80% of the time [179]. Vitamin D supplementation during pregnancy appears to reduce risk of ASD [180,181], however MTHFR status of patients taking prenatal vitamins must be known to avoid folic acid toxicity, a risk factor for ER stress [182]. The regulatory role of the Vitamin D seco-steroid (activated Vitamin D), and especially its role in healthy resolution of ERO, preventing ER hyperstress, likely explains its efficacy. Vitamin D suppresses ER stress in macrophages in type 2 diabetes [183].

Glyphosate and Many Neotoxins Induce ER Stress

A very strong temporal correlation exists between the amount of glyphosate used and rates of autism, and the ER stress response is implicated in the toxicity of glyphosate (de Liz Oliveira Cavalli et al. [184]). Like many toxins in our increasingly toxic world, glyphosate's effects are likely due to compounding effects of the need to fold intrinsically disordered proteins and widespread effects of metal ER-stress metal neurotoxicities. In addition to glyphosate, many other neotoxins in mass use are not safe for humans and other living things in that they add to the problem of ER stress and the apoptotic final solution of the UPR. These include polychlorinated biphenyl quinone according to Xu et al. [185].

Summary and Conclusions

The UPR is essential for proper proliferation, differentiation, maturation and viability of CNS cells during development (Murao and Nishitoh, 2017) and throughout life. All of the science that supports that thimerosal and aluminum (1) induce ER stress, (2) accumulate in the brain of people with ASD, and (3) are commonly injected across the entire population makes them pivotal lynchpins in setting off deranged UPRs in the face of certain genetic variants and additional parochial neotoxins. Studies that find genetic risk have been misinterpreted as "autism is genetic." It is far more likely that mutations that cause difficulty in protein folding confer risk of increased susceptibility to toxins that cause ER stress. About 1/3 of human proteins are sufficiently disordered that they require assistance

in the ER. Proteins involved in ER, mitochondria and Golgi detox pathways will confer similar risk of ER stress cellular toxicity. Exposure to toxins that induce ER stress is risky for individuals with variants that cause misfolding of proteins to be expressed. Not all proteins are expressed in all tissues, and thus environmental toxin-induced ER pathophysiologies will be expressed in different tissues in different families, accompanied by the acquisition of chemical sensitivities. In ASD, proteins that are expressed in the brain and are involved in or require ER protein folding or that are involved in ER/mitochondrial/Golgi cellular detoxification will serve as useful indicators of vaccine ASD risk (and risk of ASD from other ER-toxins). Because neurons and glial cells may not detoxify as well in these individuals, the process will also cause the retention of other, parochial toxins, consistent with the Environmental Toxin Sampling Liability model [5]. Chronic microglia activation, the specific pathophysiology most commonly seen in ASD, will occur due to ER stress-induced apoptosis, releasing cytokines and causing a re-distribution of accumulated toxins, which are then freed to induce ER toxicity in other neurons and glial cells. Aluminum cycling is especially problematic.

The link between increased risk of ASD and acetaminophen exposure after vaccination, found by a surprising number of studies, can also be explained by ER-stress and the UPR, which is the specific mechanism of acetaminophen toxicity. With the other inputs into ER stress and the UPR, the central and combined role of these factors comes into view (Figure 2). Most individuals with ASD are not born with ASD as a fixed genetic condition. The causes of the condition are going to be found to be reversible for many, if not most with ASD's. Specific mutations can be found in individuals that will be found to have difficulty folding in the ER. This knowledge can be expected to lead to immediate clinical changes. For individuals with normal Vitamin D metabolism (no LOF mutation in Vitamin D Receptor genes), increased Vitamin D for a week or two before and after vaccination should be protective. Individuals with mutations that confer risk of ER stress or problematic folding should perhaps be advised to space out vaccines, or skip some boosters, and to avoid overall exposures to mercury and aluminum from all sources.

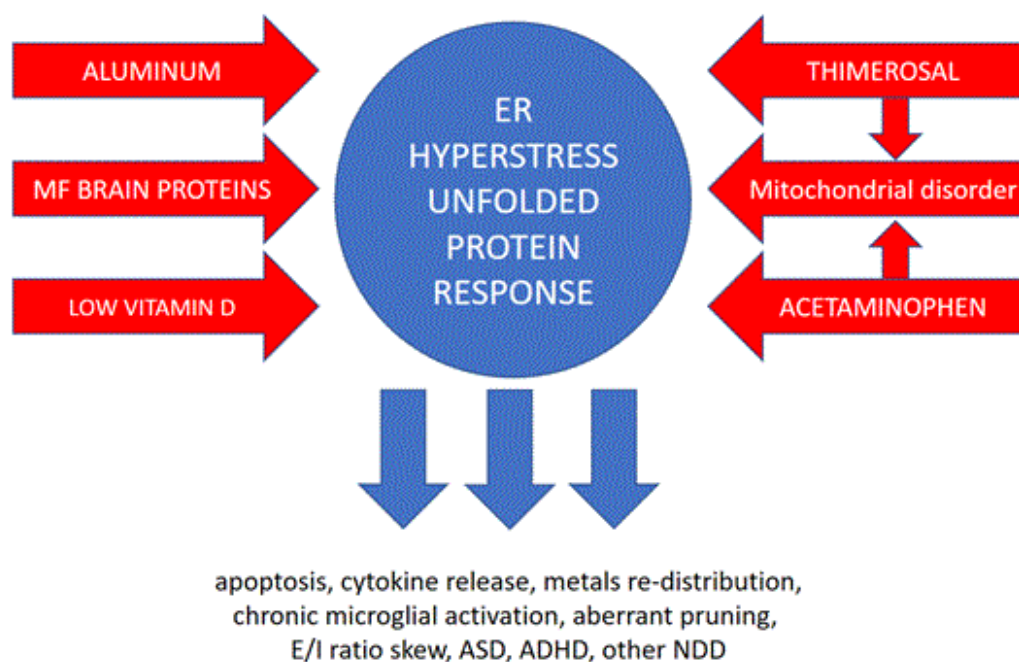


Figure 2: Multiple otherwise disjointed lines of evidence are explained via their individual and combined effects on ER stress and the unfolded protein response. MF=misfolded; E/I=excitatory/inhibitory synaptic ratio, ADHD=attention deficit hyperactivity disorder; NDD=neurodevelopmental disorders.

Vitamin supplementation will not be a panacea because some vitamin receptors will be found to be dysfunctional in some children and adults with ASD. A reduction in exposure to toxins combined with monitoring serum Vitamin D levels may be more appropriate. Roberts et al., like Boggess et al. found a diversity of pollutants increased in the serum of autistics. This is consistent with the break-down of cellular detoxification caused by ER Hyperstress.

At a societal level, vaccine risk denialism exists and persists due to the lack of ability to identify those most at risk. It is not a sustainable position. The relevant risk is to those with increased risk, not to the population as a whole. The position that vaccines do not cause autism is due to misuse of the results of studies that have failed to detect association at the population level. Such studies are not designed to and therefore cannot rule out specific risk for any individual, and not in a heterogeneous genetic minority. Vaccine risk denialism is based on fears that people will stop vaccinating if an association is admitted, even if only for a genetic minority. Focus on the effects of specific mutations on brain protein folding in the ER should lead to the ability to rapidly and accurately identify the at-risk genetic minority. With clinical exome sequencing paired with LOF and protein-folding functional interpretation focused on the ER stress response, and general risk of neurodevelopmental disorders, we can make the clinical landscape safer for everyone, raise the bar of societal "debates" on vaccine risk, protect those most at risk, and save billions of dollars in the vaccine compensation fund. It must be recognized that encephalopathies induced in some by vaccines can be variously characterized as "channelopathies", "mitochondrial dysfunction", etc. that then give rise to the ASD phenotype as neuronal and glial toxicity

and excitotoxicity accelerate. This means that vaccines may cause autism and ADHD in some people. These intermediate phases leading to ASD should not be confused with causes and outcomes.

Since a small percentage are influenced, one can predict the failure of most epidemiological studies to detect the effect at the whole-population level. Mandates for vaccinations are not ethical, as they condemn with certainty a minority of individuals to pain and suffering for the benefit of the majority. Medical clinical exome sequencing will also prove lucrative to pediatric and ob/gyn practices, the freedom of choice to vaccinate or not vaccinate can be better respected, and doctors will be able to fulfill their duty to obtain truly informed consent on risk instead of relying on overgeneralizations that place families in their care at increased risk of stresses from lifelong disability, increased cost of medical care, job loss, and divorce.

Studies are needed to determine the relative contribution of various sources of ER-stress to ASD and other neurodevelopmental disorders and the effects of avoidance of ER stressors on ASD risk. The most ethical of these studies would be interventional, (e.g., vaccination cessation in ASD) to measure the effects of avoidance of these toxins alone and in pairs to measure singular and interaction neurotoxicities, combined with vitamin supplementation and serum level monitoring of both vitamin levels and parochial pollutants that accumulate due to detoxification deficiency.

Much additional published research is consistent with the vaccine/autism hypothesis (Lyons-Weiler, 2016), which should now be formally adjusted to "Vaccines may induce autism in a genetic minority of patients".

Disclosure

The author has served as expert witness on vaccine injury cases in the National Vaccine Compensation Program. To date, he has not served as an expert witness on any cases involving ASDs.

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