Review

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2017, Vol. 45(2) 407–438

DOI: 10.1177/0300060517693423
journals.sagepub.com/home/imr
SAGE

The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism

William Parker[1], Chi Dang Hornik[2], Staci Bilbo[3], Zoie E. Holzknecht[1], Lauren Gentry[1], Rasika Rao[1], Shu S. Lin[1], Martha R. Herbert[4] and Cynthia D. Nevison[5]

Abstract

The wide range of factors associated with the induction of autism is invariably linked with either inflammation or oxidative stress, and sometimes both. The use of acetaminophen in babies and young children may be much more strongly associated with autism than its use during pregnancy, perhaps because of well-known deficiencies in the metabolic breakdown of pharmaceuticals during early development. Thus, one explanation for the increased prevalence of autism is that increased exposure to acetaminophen, exacerbated by inflammation and oxidative stress, is neurotoxic in babies and small children. This view mandates extreme urgency in probing the long-term effects of acetaminophen use in babies and the possibility that many cases of infantile autism may actually be induced by acetaminophen exposure shortly after birth.

Keywords

Autism, inflammation, oxidative stress, acetaminophen, paracetamol, paracetamolo

Date received: 26 September 2016; accepted: 20 January 2017

[1]Departments of Surgery, Duke University Medical Center, Durham, NC USA
[2]Departments of Pediatrics, Duke University Medical Center, Durham, NC USA
[3]Departments of Pediatrics, Harvard Medical School, Charlestown, MA, USA
[4]Departments of Neurology, Harvard Medical School, Charlestown, MA, USA
[5]Institute for Arctic and Alpine Research, University of Colorado, Boulder, Boulder, CO, USA

Corresponding author:
William Parker, Duke University Medical Center, Box 2605, Department of Surgery, Durham, NC 27710, USA.
Email: William.parker@duke.edu

Introduction

Autism is a complex disorder associated with a wide range of disparate and seemingly unrelated factors such as (a) maternal exposure to various chemical substances, (b) maternal exposure to child abuse, (c) maternal evidence of diabetes or other autoimmune diseases, (d) age of either parent at conception, (e) exposure of the infant to various chemical substances, (f) vitamin D levels of the infant at birth, (g) gender of the infant, and (h) a large number of genetic factors. With this in mind, we believe it is helpful to categorize the factors associated with autism in an effort to identify patterns which may be informative. As described below, these risk factors for autism fall into two primary categories: those associated with inflammation and those associated with oxidative stress.

A number of risk factors for autism can be categorized as risk factors for inflammation or indicators of inflammation (Table 1). Risk factors for inflammation associated with autism include maternal (odds ratio [OR] = 1.6, confidence interval [CI] 95% = 1.1–2.2) and perhaps paternal (OR = 1.4, CI 95% = 1.0–2.0) autoimmune diseases such as diabetes, myasthenia gravis, idiopathic thrombocytopenic purpura, or rheumatic fever,[1] maternal obesity (OR = 1.67, CI 95% = 1.1–2.56),[2] and febrile episodes during the first two trimesters (OR = 1.6, CI 95% = 1.0–2.5).[3] An increase in inflammation among individuals living in Western societies is evidenced by the alarming rise of allergy and autoimmune disease in the United States and other Westernized countries over the past century and can be attributed to five major causes that result directly from social and cultural changes: (a) an ''inflammatory diet'' high in fat and simple sugars and low in fiber and nutrients, (b) lack of physical exercise, (c) chronic and unrequited psychological stress, (d) vitamin D deficiency, and (e) biome depletion.[4–7] The recent rise in autism[8,9] could be attributable, at least in part, to this rise in inflammation.[10,11]

Aside from inflammation, oxidative stress is the second category in the landscape of autism-associated factors. As pointed out by Chauhan and Chauhan, a wide range of studies ''... suggest increased oxidative stress in autism that may contribute to the development of this disease.''[12] However, it is unknown whether factors causing oxidative stress have increased in Western societies concomitantly with inflammation. Perhaps the weight of factors causing oxidative stress peaked during the height of the industrial revolution and has since declined from that peak,[9] but this is unknown. Indeed, it may be difficult or even impossible to determine whether, in terms of oxidative stress, modern factors such as pharmaceuticals, pollutants from the combustion of fossil fuels, and other factors from industrial processes outweigh more historical factors such as smoke from cooking fires and naturally occurring toxins from food obtained by hunting and gathering. Nevertheless, oxidative stress is now associated with modern diseases. Oxidative stress, like inflammation, is associated with cancer,[13] coronary artery disease,[14] and a number of psychiatric disorders.[15] It is widely thought that inflammation and oxidative stress go hand in hand; as stated by Ghezzi and colleagues, ''The mechanism by which oxidative stress induces inflammation and vice versa is unclear but is of great importance.''[16] Therefore, either oxidative stress has increased or other changes in modern society (e.g. increased inflammation) have made oxidative stress more dangerous than it was in past generations.

Thus, the list of diverse factors associated with the induction of autism can be viewed as interrelated when examined in the context of two major categories, inflammation and oxidative stress (Figure 1). For the purpose of this discussion, factors involved in the

Table 1. Risk factors associated with autism, in very approximate descending order of risk.

Factor	Classification
High Risk	
Acetaminophen use in children[a]	Pharmaceutical: oxidative stressor, decreased capacity to handle oxidative stress
Excessively high levels of vitamin B12 and folate in maternal blood	Linked to inflammation and possibly to oxidative stress
Down's Syndrome	Oxidative stress
Preterm delivery	Risk factor for oxidative stress and indicator of inflammation
Cerebral palsy	Indicator of inflammation
Environmental toxins: pesticides	Oxidative stressors, inflammatory stimuli
Male gender	Risk factor for oxidative stress, susceptibility to oxidative stress
Maternal exposure to childhood abuse	Risk factor for inflammation later in life
Hepatitis B vaccine, first month of life, pre-1999	Oxidative stressor, inflammatory stimulus
Polymorphic variants (various, involved in methionine and glutathione pathways)	Decreased capacity to handle oxidative stress
Mother with diabetes	Indicator of inflammation
Mother with lupus	Indicator of inflammation
Moderate Risk	
Father >50 years old	Risk factor for inflammation
Parental autoimmune disorder	Indicator of inflammation
Maternal obesity	Risk factor for inflammation
Hyperbilirubinemia	Risk factor for oxidative stress
Early childhood atopic disorders (dermatitis, respiratory)	Indicator of inflammation
Maternal autoimmune disease	Indicator of inflammation
Febrile episode >7 days	Symptoms associated with oxidative stress and inflammation
Use of acetaminophen during pregnancy	Pharmaceutical, oxidative stressor
Infection during pregnancy	Oxidative stressor, inflammatory stimulus
Low or Uncertain Risk	
Mother smoking during pregnancy	Oxidative stressor
Mother >40 years old	Risk factor for inflammation
Environmental toxins: air pollution, including vehicular emissions of heavy metals and particulate matter	Oxidative stressors, inflammatory stimuli
Aspartame/other sources of methanol	Oxidative stressor
Low vitamin D levels at birth	Inflammatory mediator
Folate deficiency (anti-folate receptor autoantibodies)	Risk factor for oxidative stress
Urbanization/Western society	Risk factor for inflammation

Citations are in the text.
[a]Determined only in a single, survey-based study.

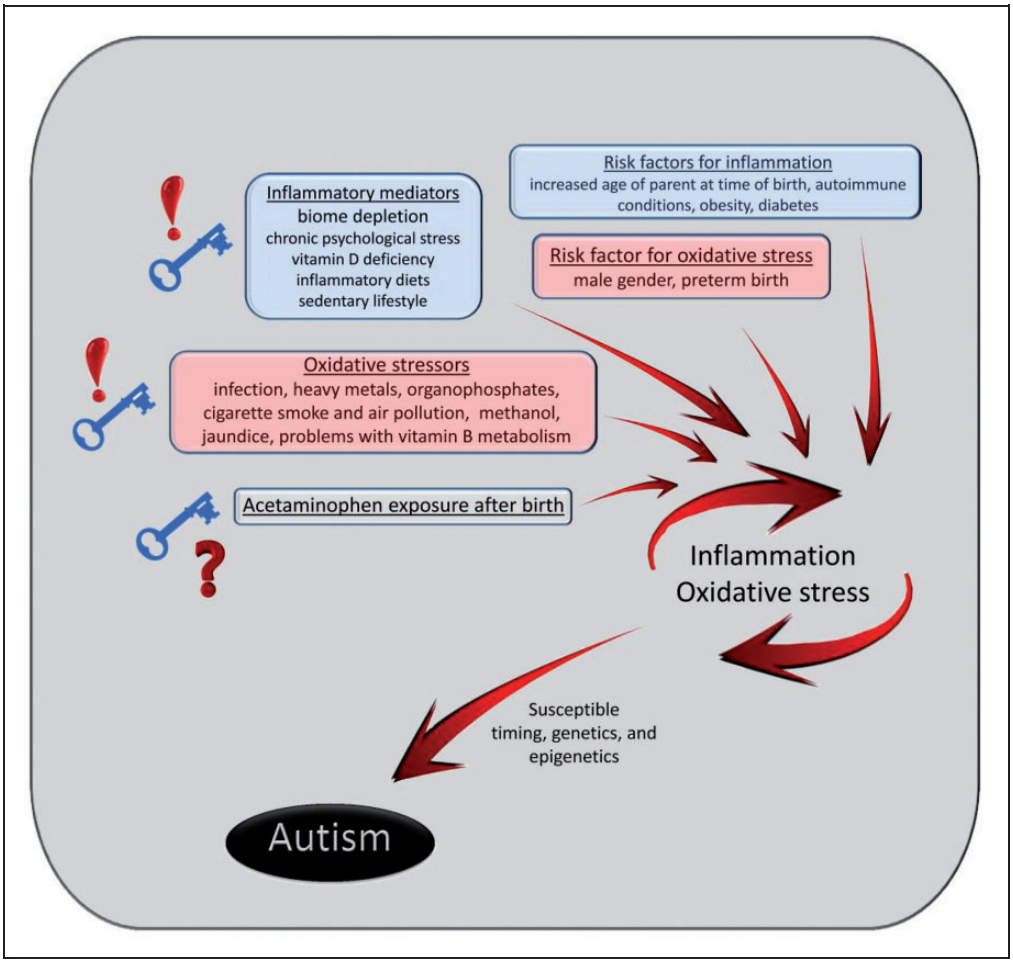

Figure 1. The role of oxidative stress (red), inflammation (blue), and possibly acetaminophen exposure after birth in the induction of autism.

induction of autism will be described as "low risk" (1%–20% increased risk of autism), "moderate risk" (21%–100% increased risk of autism), or "high risk" (any risk of autism above 100% or 2-fold).

Autism and inflammation

Several risk factors found for autism have very straightforward connections to inflammation (Table 1). Episodes of fever greater than 7 days in the first and second trimester carry a moderate risk factor for autism (hazard ratio [HR] = 1.6, CI 95% = 1.0–2.5).[3] In addition, autoimmune diseases in the mother such as lupus, multiple sclerosis, diabetes, and rheumatic fever are associated with moderate-to-high risk for autism induction,[17] as is maternal infection during pregnancy.[18] Further, atopic dermatitis and respiratory diseases in children under the age of 2 years are also associated with a moderate risk for the development of autism.[19]

Another moderate risk factor for autism that has a well-known association with

inflammation is obesity (OR = 1.67; CI 95% = 1.1–2.56).[2] Over one-third of women in the United States of childbearing age are obese and this number is steadily rising.[20] Multiple studies link obesity to chronic, low-grade inflammation and the subsequent secretion of pro-inflammatory cytokines and infiltration of immune cells.[21] Although it may be tempting to blame the rise in autism on the rise in obesity and autoimmune disease, these risk factors alone, which are generally associated with low-to-moderate increases in autism prevalence, likely do not account for the 30-fold or greater increased prevalence of autism since the 1970s.[9,22]

The prevalence of autism in children with cerebral palsy is approximately 7-fold greater than in the general population.[23] Substantial evidence points toward the view that cerebral palsy, the most common cause of severe motor disability in childhood, is associated with inflammation. For example, many studies have linked fetal exposure to infection to the development of cerebral palsy,[24–26] and blood samples from neonates with cerebral palsy contain higher concentrations of many pro-inflammatory cytokines and chemokines compared with controls.[27] Further, cerebral palsy is associated with chorioamnionitis,[26,28–30] which can stimulate fetal production of inflammatory cytokines including IL-6.[31] Thus, cerebral palsy, a high risk factor for the development of autism, is linked to inflammation.

Some risk factors for autism have less obvious associations with inflammation. For example, increasing age of the mother or the father at the time of birth is associated with a low or moderate risk of autism, respectively (OR = 1.15, >40 years for the mother and OR = 1.66, >50 years for the father).[32,33] While age itself might not normally be considered inflammatory, there have been direct associations found between increased age of the parents and inflammatory diseases in their offspring. For instance, maternal age ≥30 years at the time of birth is associated with an increased incidence of one or more food allergies in the child compared with controls (75% vs. 55%, p = 0.005).[34] Further, the risk of having children diagnosed with multiple sclerosis steadily increases with paternal age at birth from 21 to 55 years (adjusted OR for 21–25-year-old fathers = 1.08 and 51–55-year-old fathers = 2.00).[35] Thus, although parental age, a moderate risk factor for autism, is not intuitively associated with inflammation, the association is evident based on published studies.

Maternal exposure to severe emotional stress during her childhood and/or adolescence is another risk factor (OR = 3.7, CI 95% = 2.3–5.8) for autism[36] that may not be intuitively associated with inflammation. However, the link between childhood and adolescent adversity and immune dysregulation and inflammation is widely appreciated by the scientific community. Multiple studies demonstrate that a wide range of stressful events taking place in one's childhood can adversely affect health and inflammatory responses even into the eighth decade of life.[37–40] Exposure of women to two or more early childhood stressful events, including physical, emotional, or sexual abuse, is positively correlated with an increased risk of Th1, Th2, and rheumatic autoimmune development later in life compared with women reporting no events in childhood ($p < 0.05$).[41] Markers of inflammation (IL-6 and C-reactive protein) have been found to be elevated in a systemic manner in women who experienced sexual abuse in adolescence compared with women without a history of abuse (p = 0.04 for IL-6 and 0.03 for C-reactive protein).[42] Further, a recent study of 28,456 African American women found a positive association between adult-onset asthma and physical or sexual abuse experienced during childhood or adolescence after adjusting for multiple confounders such as parental history of asthma, body mass index, exercise, current smoking

habits, and exposure to secondhand smoke.[43] Childhood stress also affects a woman's response to current, daily stressors. For example, elevated levels (2.35-fold) of IL-6 have been found within 24 hours in response to current daily stressful events in sera from women who experienced childhood abuse compared with women who experienced the same number of daily stressors without a history of child abuse.[44] Other examples of the impact of early life stress on inflammation abound.[37,45–48] Thus, maternal exposure to stress during early life, a high risk factor for autism, is clearly associated with inflammation.

Autism and oxidative stress: Environmental toxins

Exposure to environmental toxins, a source of oxidative stress, has been associated with autism in a number of studies.[49] Maternal exposure during gestation to agricultural pesticides such as organophosphates, organochlorines, and pyrethroids has been identified as a moderate-to-high risk factor for autism.[50–52] The risk seems to be dependent at least in part on the proximity to the applied chemical and the trimester during which the exposure took place. However, the data are somewhat noisy and, as pointed out by the authors, possibly confounded by unavoidable misclassifications in the estimations of maternal exposure. In contrast to evidence of the effects of exposure in utero, evidence for exposure to pesticides after birth as a risk factor for autism is slim to non-existent. Maternal exposure to traffic-related air pollutants also carries risk factors for autism. For example, exposure to particulate matter during the third trimester is a low-to-moderate risk factor for autism,[53–55] while maternal exposure to other traffic-related toxins (e.g. mercury, lead, arsenic, cadmium, manganese, styrene, trichloroethylene, and vinyl chloride) is a moderate-to-high risk factor for autism.[56–58] However, these findings were not replicated in a study of four European countries.[59] Furthermore, the range of variability in U.S. air pollution (e.g. interquartile ranges of only 4–5 mcg/m^3 in fine particulate matter [PM2.5]) over which associations with autism spectrum disorder have been reported[53–55] is quite small compared with the more severe air pollution encountered routinely in the developing world (for example, >900 mcg/m^3 when indoor cooking stoves are used).[60] Additionally, exposure to many environmental toxins such as vehicular and air pollution, polychlorinated biphenyls (PCBs), lead, polycyclic aromatic hydrocarbons (PAHs), and organochlorines and organophosphates has decreased over at least part, if not most, of the time frame in which autism prevalence has continued to climb.[9] Further, factors such as exposures to phthalates, atmospheric mercury levels, and total blood mercury have remained relatively stable since the 1990s, although exposure to polybrominated diphenyl ethers (PBDEs) and glyphosates has increased.[9] However, to date, few studies have associated exposure to phthalates, PCBs, PBDEs, PAHs, or glyphosates to the development of autism. With this in mind, it seems likely that the relative contribution of any single environmental oxidative stressor to the prevalence of autism is most likely dependent on the location or population in question.

Autism and oxidative stress: Jaundice

Hyperbilirubinemia (the symptoms of which are known as jaundice) is associated with elevated oxidative stress[61] and impedes the clearance of acetaminophen,[62] a drug suspected of inducing autism (see discussion below). Jaundice is associated with autism,[63] although the connection between jaundice and autism had some early critics.[64] The idea was that early studies pointing toward a connection between jaundice and autism

may have been confounded by some condition or conditions associated with autism but unrelated to jaundice. These conditions may have been responsible for keeping the children in the hospital for longer periods of time, which could have resulted in an increased chance of receiving a diagnosis of jaundice. However, a prospective study design using a large sample size in Taiwan seems to have addressed major concerns with the previous studies, and the connection between jaundice and autism is apparently real.[65]

Autism and oxidative stress: Sex, Down syndrome, and preterm birth

Perhaps the most widely known risk factor for autism is being male. The increased risk of autism for males was established very early during work with patients having autism, and is approximately 4-fold that of females. Although being male is not widely known to be associated with oxidative stress, male infants are more susceptible to oxidative stress than female infants,[66–68] and have been found to have more oxidative stress.[69] Further, male children seem to be more susceptible to toxin-induced oxidative stress than females.[70] The occurrence of Down syndrome is also a risk factor for autism, with more than 5% of individuals with Down syndrome having autism.[71] Down syndrome is also associated with a profound increase in oxidative stress and inflammation,[72,73] pointing again toward a connection between oxidative stress and the induction of autism.

Preterm birth, a complex and often idiopathic complication of pregnancy, is a high risk factor for the development of autism. Limperopoulos and colleagues found that 26% of preterm, low birth weight infants had a positive result on the Modified Checklist for Autism in Toddlers (M-CHAT) autism screening tool[74] compared with 5.7% of non-preterm infants (OR=4.56).[75] This risk is corroborated by several additional studies with odds ratios of 6.3 (CI 95% = 2.2–18.3) for children born preterm in the United Kingdom and Ireland[75] and 3.2 (CI 95% = 2.6–4.0) for children who were born preterm in Sweden using a sibling-comparison approach.[76] Preterm birth has long been associated with inflammation, with approximately 50% of preterm births being associated with chorioamnionitis.[77,78] In addition, oxidative stress has recently been hypothesized as a co-mechanism for the initiation of preterm birth.[77,79,80] Further, oxidative stress is associated with preterm birth.[81–83] Thus, preterm birth, a risk factor for autism, is associated with both inflammation and oxidative stress.

Autism and oxidative stress: Vitamin B

Excessively high levels of maternal vitamin B12 and vitamin B9 (folate) are additional risk factors linked to autism.[84] In a study of the association between vitamin B levels and risk of autism, excessively high prenatal vitamin B12 levels in mothers (HR = 3.01, CI 95% = 1.64–5.52; *P* value: 0.001) and excessively high prenatal folate levels (HR=2.27, CI 95% = 1.26–4.09; *P* value: 0.007) were each found to be associated with a significantly increased risk of infant autism. Together, excessively high vitamin B12 and folate levels in maternal blood sera show the highest increased risk of infantile autism (HR: 17.59; *P* value: <0.001).[84]

Vitamin B9 and B12 are antioxidants, and thus, intuitively, high levels of these vitamins should be associated with a decreased risk for oxidative stress. However, excessively high levels of vitamin B12 in the blood are common and are often paradoxically indicative of a clinical deficiency of vitamin B12.[85] In one case, for example, excessively high levels of vitamin B12 were caused by the presence of anti-vitamin B12 antibodies that led to the formation of functionally inactive IgG-IgM-B12 immune complexes.[86] In

general, excessively high levels of vitamin B12 and the associated functional deficiency of that antioxidant could lead to difficulties in the ability to cope with oxidative stress, and indeed have been consistently linked to inflammatory diseases such as neoplasms, hematological malignancies, and liver and kidney diseases.[85]

Not only are exceedingly high levels of folate associated with autism,[84] the presence of anti-folate receptor autoantibodies has been shown to be highly prevalent (75.3%) in children with autism.[87] Such autoantibodies would create a functional deficiency of folate in the brain regardless of the concentration of folate in the serum. Folate deficiency is highly deleterious and is associated with reduced activity of antioxidant enzymes, as well as overall increased oxidative stress.[88,89]

Hypothetically, excessive levels of folate in the serum could be an indication of a clinical insufficiency of folate, just as excessively high levels of vitamin B12 are linked to a clinical insufficiency of that vitamin. Regardless of the reasons for excessively high levels of folate, they have been associated with inflammatory bowel disease,[90] indicating that the high levels are related in some way to inflammation.

Autism and oxidative stress: Genetic variation

Polymorphic variants related to the metabolism of methionine transmethylation and transsulfuration, which increase susceptibility to endogenous and environmental oxidative stress, are significantly different in children with autism.[91–93] Studies show a decreased ability in children with these genetic variants to handle oxidative stress as measured by several metabolic biomarkers including S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH), adenosine, homocysteine, cystathionine, cysteine, oxidized and reduced glutathione, endogenous secretory receptor for advanced glycation end-products (RAGE), and the pro-inflammatory ligand S100A9. In addition, polymorphisms in glutathione pathways, which modulate the response to oxidative stress, strongly affect risk for autism. For example, the homozygous GSTM1 deletion genotype imposes a near 2-fold increased risk for autism.[94,95] Further, polymorphisms of the glutathione S-transferase P1 gene (*GSTP1*) in the mother, which could affect the fetus during pregnancy, are high risk factors for the induction of autism (OR = 2.67, CI 95% = 1.39–5.13).[96] Thus, several genetic variants that affect pathways involved in oxidative stress are risk factors for autism.

Synergism between oxidative stress and inflammation: Evidence from animal models

Inflammation can be caused by a diverse set of factors associated with Western culture, including chronic psychological stress and biome depletion (Figure 1), which at first glance might seem unrelated to inflammation. However, it is now recognized that these non-chemical inflammatory mediators (e.g. limited resources or social support for the mother, leading to chronic psychological stress) can increase vulnerability of the fetus to chemical stressor exposures (e.g. oxidative stressors such as pollution or toxins).[97] This view potentially explains why a single exposure or risk factor in isolation is a modest predictor of autism risk. Given the complex nature of environmental and social exposures, attempts at deciphering the mechanisms that contribute to autism suffer from fatal oversimplification if those models involve only single agents. In contrast, useful and relevant models must include multiple factors. For example, an experimental animal model has been developed that employs the combined effects of an ethologically relevant maternal stressor and an environmentally relevant pollutant, diesel exhaust, both of which have been

implicated in autism.[55,57,98–102] Using this model, it was demonstrated that maternal exposure to diesel exhaust particles combined with maternal stress, *but neither in isolation*, produced long-term cognitive deficits and strikingly increased anxiety in male but not female offspring.[97]

Evidence for a mediator other than inflammation and oxidative stress

The likely role of oxidative stressors and inflammation in the pathogenesis of autism is apparent based on the nature of risk factors associated with autism. This conclusion is corroborated by numerous studies, described above and reviewed elsewhere,[10,103–109] that have identified immune activation and inflammation in patients with autism. Further, treatment with antioxidants or anti-inflammatory agents such as sulforaphane[110] and helminth therapy,[111] respectively, can help some patients with autism. However, it seems unlikely that oxidative stress and inflammation alone account for the dramatic rise in the incidence of autism since 1980. Inflammation in general has increased steadily since the turn of the twentieth century, as indicated by a slow and steady rise in a wide range of allergic disorders, autoimmune conditions, and other inflammation-associated diseases.[112] The very rapid rise in autism since the early 1980s might suggest that one or more specific environmental factors are at play. As we and others have pointed out,[9,10,113] it is possible that the rapid "increase" in autism is due simply to increased awareness and changing diagnostic criteria, but substantial evidence which contradicts this conclusion is available and a working hypothesis that autism has profoundly increased over the last 40 years holds the most promise for a rapid resolution of the problem.[10] That is to say, if autism is indeed an ancient and natural consequence of "being human," then autism may be much more difficult to prevent than if it is induced by factors present in modern society.[10]

We have suggested[10] that arguments for and against autism as an epidemic (or pandemic) can only be resolved (a) if a specific trigger for autism is found, facilitating the elimination of autism; or (b) if complete normalization of the immune system (leading to a pre-industrial condition of essentially no allergies) succeeds in eliminating autism. Given that complete normalization of the immune system may take years or even generations to accomplish, and given the recent apparent rise in autism described above, the search for very specific and potent triggers for the induction of autism seems worthwhile.

Proposed triggers of the autism epidemic: Aspartame

Aspartame is an artificial sweetener that has been in use since 1981, about the time that the autism epidemic started (Figure 2). Aspartame breaks down in the body and releases methanol, an oxidative stressor and toxin. Its use has been attributed to the rise in autism.[114] However, the use of other sweeteners has replaced aspartame in the past 10 years, except in diet soft drinks, and the consumption of diet soft drinks has been in decline. Thus, there is no association over time between autism and aspartame. Further, the presence of methanol in canned vegetables and in cigarette smoke is not new, and thus the relatively low incidence of autism prior to the mid-1900s cannot be accounted for if methanol, by itself, is a major trigger. Further, Pepsi Cola® has at least temporarily removed aspartame from its drinks, which eliminates at least for a time one of the major remaining sources of aspartame consumption in the United States. If indeed aspartame is the major trigger for autism, then the "experiment" has been accomplished already and

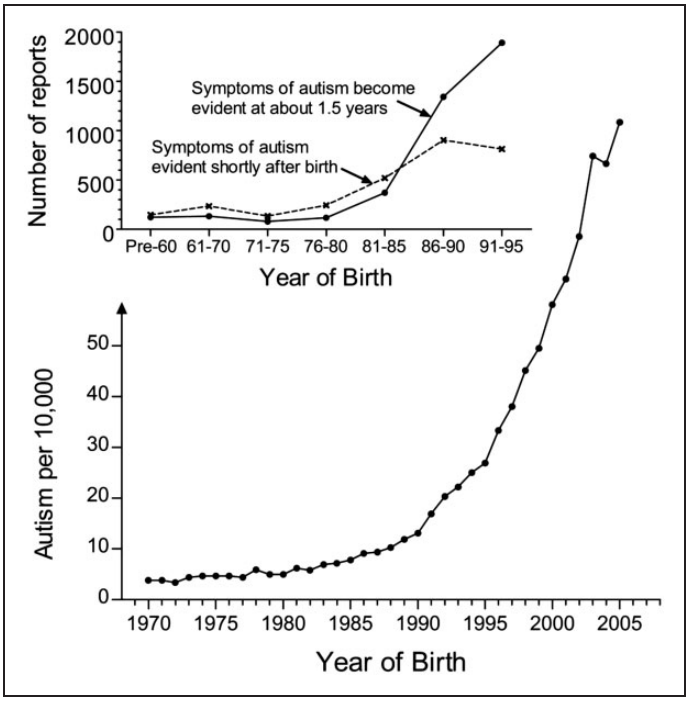

Figure 2. Apparent changes in the quality and quantity of autism extending over a decade, starting in the early 1980s. In the **top diagram**, data are from Rimland's summary[168] of the number of surveys (the "E-1 Diagnostic Checklist" and the "E-2 Diagnostic Checklist") that were collected in a given time period through grass-roots efforts of the Autism Research Institute and the Autism Society of America, the only two national autism organizations in the United States at the time of the data collection. The Y-axis describes the actual number of surveys received, and changes in the number of reports received were attributed by Rimland to increases in the number of children with autism. "Shortly after birth" in this case refers to parents' reports that symptoms of autism were evident within weeks of birth. In the **lower diagram**, the prevalence of autism in California as compiled by Nevison[9] is shown. Data are a composite of "snapshot" data (information collected at one point in time) from the California Department of Developmental Services (collected in 2002 and covering birth years 1970–1997) and tracking data evaluating 5-year-olds collected under the US Individuals with Disabilities Education Act (covering birth years 1995–2005).[9]

the rate of autism should be in the process of decreasing. This does not appear to be the case, apparently disproving the hypothesis.

Proposed triggers of the autism epidemic: Ethyl mercury and vaccines

The view that vaccines and ethyl mercury in particular, a component of the preservative thimerosal used in some vaccines, can induce the development of autism is widespread among non-scientists[115,116] and has been discussed widely in the literature.[117,118] Proposed mechanisms of induction depend on the difference between methyl and ethyl mercury[119] to account for the relatively recent rise of autism compared with the centuries-old use of methyl mercury. However, a study addressing this issue found no association between levels of mercury exposure during vaccination and

the incidence of autism 120. Further, a substantial reduction in exposure to ethyl mercury as a result of elimination of thimerosal from vaccines has not reduced the rate of autism. It has been counter-argued that "no level is safe," suggesting that any amount of ethyl mercury may be dangerous. However, since the induction of autism (by whatever agent) is apparently not near the saturation point (presumably yielding a rate of 100% autism in the population, at which point the rate of autism would be independent of increasing or decreasing levels of inducing agent), then it is expected that a substantial reduction in the amount of inducing agent would lead to at least some reduction in the level of autism. It has been argued that perhaps aluminum adjuvants now replace mercury preservative as the vaccine-associated agent, but then ethyl mercury and aluminum must be different from methyl mercury in some regard, straining the original hypothesis regarding the uniqueness of ethyl mercury. It could be counter-argued that the route of exposure (injection vs. dietary intake) of the metal is important, but in the one widely accepted instance in which a vaccine caused the induction of a neuropsychiatric disorder (narcolepsy with cataplexy), no metal preservatives or metal adjuvants were involved.[120] Thus it could be argued that it is the vaccination in general that is critically important, not the metal per se. Yet vaccines are much older in their origins than autism, and it is not intuitive that a vaccine would be worse than an actual life-threatening infection, the origins of which are ancient and pre-date the human race. This view is corroborated by a cohort of parents who did not vaccinate younger siblings of children with autism; failure to vaccinate with one or even all vaccines did not prevent autism.[122,123] Most importantly, acetaminophen, the analgesic most commonly administered in conjunction with vaccination and the only analgesic administered to children under the age of 6 months following vaccination, has been identified as a likely inducer of autism.[124] Initial studies described below suggest that it is the co-administration of this analgesic with vaccines that may have given many parents the false impression that their child's autism was induced by a vaccine.

Proposed mediators of the autism epidemic: Acetaminophen

Acetaminophen has been widely used in adults for more than half a century as a pain reliever and anti-pyretic, and is by far the most commonly used medication for pain and fever during pregnancy[125] and in the pediatric population.[126,127] By the early 1980s, acetaminophen had effectively replaced the analgesic phenacetin, which is metabolized to acetaminophen by the body[128,129] and which had been widely used since the late 1800s despite its carcinogenic and toxic nature. The therapeutic action of acetaminophen involves inhibition of prostaglandin synthesis, a surprisingly important biochemical process involved in development and neurological function, as shown in Figure 3. Acetaminophen elimination from the body typically involves biochemical modification in the liver by phase II metabolism, which entails the addition of sulfate (or glucuronide more often in adults[130]) to the molecule, facilitating its elimination (Figure 3). However, the drug can also be modified via phase I metabolism, producing NAPQI, a highly toxic metabolite which is then processed via phase II metabolism to a non-toxic product by the addition of cysteine in a manner dependent on glutathione (Figure 3). Acetaminophen is known to be safer in children than in adults, presumably because the drug cannot be rapidly converted by the child's relatively undeveloped liver into toxic metabolites.[131] However, this view of acetaminophen's safety is based strictly on the low rate of

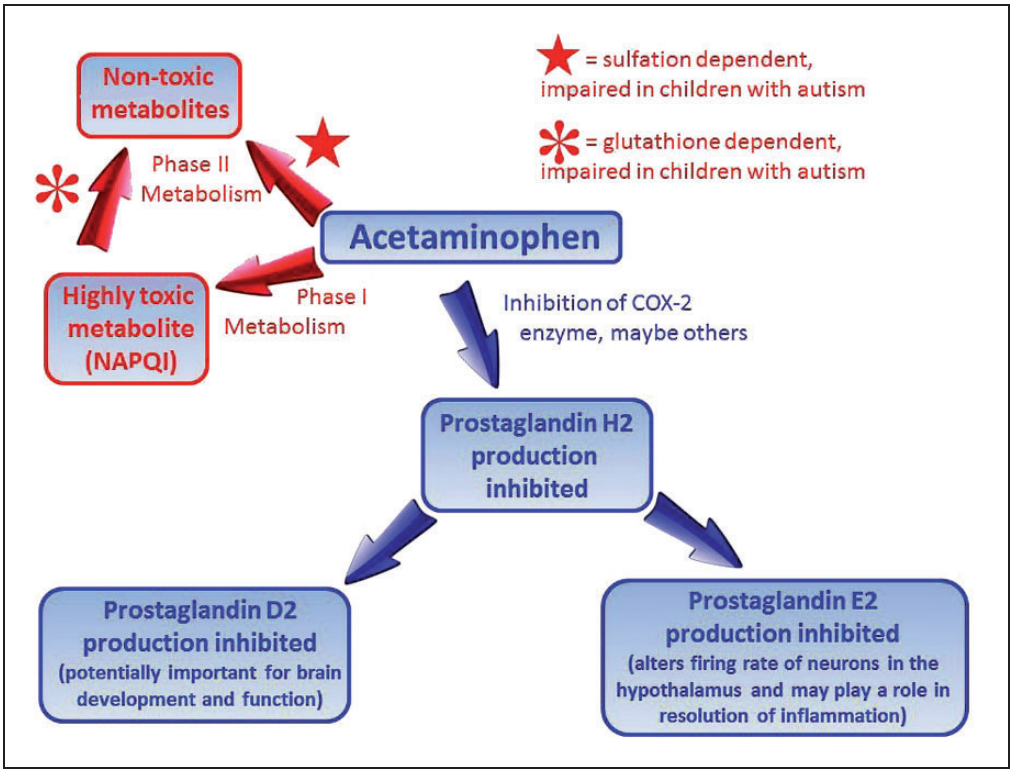

Figure 3. Action and metabolism of acetaminophen in babies and children. Phase II metabolism involving glucorinadation, like sulfation, leads to detoxification of acetaminophen, but sulfation is the primary mechanism active in infants and children.

acute adverse events such as liver and kidney failure and intestinal bleeding.[131,132] Unfortunately, the long-term effects of acetaminophen exposure on neural development have never been evaluated in humans. However, Margaret McCarthy's lab has shown that drug-mediated inhibition of prostaglandin synthesis (as is accomplished by acetaminophen) in laboratory rats during "a time sensitive window in early postnatal life" not only results in significant long-term modifications to brain development and morphology but also leads to decreased social interactions and reduced sensory function in male but not female animals.[133] Further, in vitro studies using human cell lines have shown that acetaminophen can cause "an immediate, reversible, dose-dependent loss of oxygen uptake followed by a slow, irreversible, dose-independent death" and have suggested mechanisms by which acetaminophen may cause toxicity in tissues other than the liver.[134]

Given its suppression of the febrile response, one might expect that the general effects of exposure to acetaminophen would be anti-inflammatory in nature. Unfortunately, this expectation is false. Studies in adult humans demonstrate that even low-dose acetaminophen triggers immune system activation and oxidative stress responses.[135] Further, medical professionals evaluating immune responses from a biologists' perspective have warned that the

febrile response is both adaptive (beneficial to survival) and ancient in its origins,[136] with behaviors which induce fever-like temperatures evident in cold-blooded animals.[137] Thus, biologists argue that inhibition of the febrile response is likely not without costs.[137] It has even been postulated that the elimination of fevers may be responsible in part for the induction of autism.[138] However, the health costs of eliminating low-grade fevers in general are not known. On the other hand, some data regarding the health costs of using acetaminophen are described in the literature. A multinational study with more than 200,000 children found a dose-dependent association between use of acetaminophen in the first year of life and the occurrence of inflammatory diseases such as asthma, rhinoconjunctivitis, and eczema later in life.[139] The causal relationship between use of acetaminophen and the occurrence of asthma has been questioned,[140] but the consensus is that a relationship exists[141,142] and a population-wide increase in asthma of more than 40% may be caused by the use of acetaminophen.[143]

Perhaps even more concerning than studies demonstrating acetaminophen-induced social impairment in animal models, acetaminophen-induced injury to cultured cells, and acetaminophen-induced inflammation in adults, are studies pointing toward a connection between acetaminophen and neurological problems in children. A summary of such studies is shown in Table 2. A sibling-controlled study with over 48,000 children in Norway showed that the use of acetaminophen but not ibuprofen by mothers during pregnancy was associated with problems in the psychomotor, behavioral, and temperamental development of children at 3 years of age.[144] Further, a study in Bristol, United Kingdom, of more than 7000 children showed that maternal use of acetaminophen during pregnancy was associated with hyperactivity and ''emotional symptoms'' at age 7.[145] In addition, a study at UCLA in collaboration with scientists in Denmark and Taiwan found that children whose mothers used acetaminophen during pregnancy were at higher risk of being diagnosed with hyperkinetic disorder (HR = 1.37, CI 95% = 1.19–1.59). To quote the authors, ''Results did not appear to be confounded by maternal inflammation, infection during pregnancy, the mother's mental health problems, or other [variables they examined].''[146] A more recent Danish study came to the same conclusions.[147] Further, a study from New Zealand found associations between acetaminophen use during pregnancy and attention-deficit/hyperactivity disorder (ADHD) at 7 and 11 years of age,[148] with the authors concluding that their work supports ''earlier claims that findings [of increased ADHD with acetaminophen use] are specific to acetaminophen.'' The authors further state that ''The finding that even low doses of acetaminophen (indicated by the number of weeks of drug exposure) can affect behavior 7 years later is alarming because acetaminophen (paracetamol) is the most commonly used antenatal drug.'' Indeed, new studies supporting the view that prenatal acetaminophen use is associated with long-term negative effects on brain function are currently being published on a monthly basis.[145,149]

Acetaminophen rapidly enters the cerebrospinal fluid to exert its effects.[150] In addition to reducing fever and physical pain, acetaminophen has a profound effect on adult brain function, blunting the response to both negative and positive stimuli, including threatening stimuli,[151,152] and reducing behavioral responses to social rejection.[153] Further, the drug impairs the ability of adults to identify errors made during the performance of simple tasks.[154] In animal models, the use of acetaminophen during development has been shown to cause permanent alterations in cognitive function.[155] Despite these effects on

Table 2. Published studies probing the effects of acetaminophen on neuropsychiatric function.

Study Group	Controls or Variables Considered (other than acetaminophen use)	Symptoms	Odds Ratio
1. 163 US children, aged 5 years or less[a]	Ibuprofen use used as a control. Confounders included maternal and paternal demographics, and child's health, age, and sex.	Autism	6.1[155]
2. 48,631 Norwegian children, aged 3 years	Ibuprofen use used as a control. Confounders included maternal demographics, health (infections, fevers, pain), use of other medications, alcohol use, tobacco use, and psychological stress.	Problems in psychomotor, behavioral, and temperamental development	1.51 to 1.69[143]
3. 64,322 Danish children, aged up to 15.6 years	Confounders included maternal demographics, health (fever, infection, joint and muscle diseases), use of other medications, alcohol use, tobacco use, psychological conditions, and child's age and sex.	Autism accompanied by hyperkinetic symptoms	1.51[123]
4. 1491 Danish children, aged 5 years	Confounders included maternal demographics, health (infections, fevers, pain), ibuprofen use, aspirin use, alcohol use, tobacco use, psychological conditions, and child's sex.	Impaired attention and executive function	1.5[146]
5. 7796 British children, aged 7 years	Maternal postnatal and partner acetaminophen use used as controls. Confounders included maternal demographics, health (infections, fevers, pain), genetic risk factors, alcohol use, tobacco use, psychological stress, and child's birth weight and gestational age.	Hyperactivity and "emotional symptoms"	1.29 to 1.46[144]
6. 64,322 Danish children, aged 7 years	Confounders included maternal demographics, health (infections, fevers, pain), alcohol use, tobacco use, psychological stress, and child's birth weight and sex.	Hyperkinetic disorder and ADHD[b]	1.13 to 1.37[145]
7. 871 New Zealand children, aged 7 and 11 years	Anti-inflammatories, aspirin-based painkillers, antacids, and antibiotic use used as controls. Confounders included maternal demographics, health (fever and inflammation), alcohol use, psychological stress, and medications for psychological conditions.	ADHD	Odds ratio not calculated: approximately 10% difference in ADHD assessment[147]
8. 2644 Spanish children, aged 5 years	Confounders included maternal demographics and health (fever, infection, chronic illness), parental mental health, and child's health, age and sex.	Autism	Odds ratio not calculated: difference in CAST[c] P value = 0.006[161]

[a]This study was the only study that evaluated acetaminophen exposure during childhood. The rest of the studies evaluated the effects of acetaminophen exposure during pregnancy.
[b]ADHD; Attention-deficit/hyperactivity disorder
[c]CAST; Childhood Autism Spectrum Test

neurological function in adults, the widely appreciated connections between acetaminophen use during pregnancy and neurodevelopmental problems, and studies in animal models pointing toward potential problems with acetaminophen for brain development,[133,155] acetaminophen is now the most widely used medicine in the pediatric population, readily available for infants in an over-the-counter form labeled as "safe, gentle, and effective," with no warnings of side effects other than allergic reactions.

A connection between acetaminophen and autism was first identified in 2008 by Schultz et al.,[156] who found that acetaminophen use by children was significantly associated with autism in children aged 5 years or less (OR=6.11, CI 95% = 1.42–26.3). Subsequently, several investigators noted that the marked increase in autism, asthma, and ADHD in the early 1980s corresponded with the replacement of aspirin with acetaminophen.[157,158] In addition, Schultz noted that the long-term, steady increase in the prevalence of autism was punctuated by short-term decreases coinciding with widely publicized cases of acetaminophen poisoning that temporarily deterred the public from using the drug.[159] Further, evidence has surfaced indicating that neural pathways affected by acetaminophen may be "different" in some regards in people with autism.[159,160] This observation is potentially a "smoking gun," suggestive of the role of acetaminophen in the pathogenesis of autism. Interestingly, Bauer and colleagues noted that acetaminophen use with circumcision may be associated with an increased prevalence of autism in some locations.[157] A second and more recent study looking at the connection between circumcision and autism, this one by Frisch and Simonsen, found a 2-fold increased risk of autism identified before age 5 in circumcised boys compared with uncircumcised boys.[161] But of course, the degree to which acetaminophen use during circumcision is associated with autism will depend not only on the ability of acetaminophen to induce autism but also on other factors such as the relative amount of acetaminophen used following circumcision compared with other uses in the pediatric population.

A Danish National Birth Cohort study recently found that prenatal use of acetaminophen is associated with an increased risk of autism accompanied by hyperkinetic symptoms (HR = 1.51; CI 95% = 1.19–1.92).[124] More recently, a Spanish study supported the connection between acetaminophen exposure during pregnancy and autism in the offspring.[162] Although the increase from prenatal exposure is statistically significant and indeed concerning, the risk was more than 10-fold less than that originally identified by Schultz when evaluating the use of acetaminophen in children. As discussed below, post-partum (infancy and early childhood) exposure to acetaminophen may result in a much higher risk of developing autism than prenatal exposure.

Schultz, whose 2008 study was the first to identify acetaminophen use in small children as a potential cause of autism, very recently found that *older* children with autism actually use less (not more) acetaminophen than neurotypical controls.[163] Thus, small children who eventually develop autism have more often been exposed to acetaminophen than controls, but older children with autism are less likely to use the drug. Schultz suggested that this observation may be hypothetically explained if parents noticed that their child with autism did not respond to acetaminophen. Studies in animal models have shown that early life exposure to acetaminophen causes a lack of response to the same drug later in life,[155] and Schultz postulated that the same may occur in humans.[163] Another explanation, not mutually exclusive, is that parents may have learned that acetaminophen is associated

with autism, and thus discontinued administration of the drug to their child. The connection between acetaminophen and autism is common knowledge among parents who have children with autism, as it has been promoted by grass-roots organizations such as Reset.Me and SafeMinds.

Plausible mechanisms for the induction of autism by acetaminophen have been formulated,[113,158,159] adding further support to the potential role of acetaminophen in the pathogenesis of autism. McCarthy, for example, has provided a plausible explanation for the idea that inhibition of prostaglandin synthesis (by acetaminophen, for example) would have a much different effect on the male brain than on the female brain.[164] However, the existence of a plausible mechanism alone may be considered inadequate by itself to support studies regarding the acetaminophen–autism connection, since detailed mechanisms have been proposed for the induction of autism by several factors, including ethyl mercury from vaccine preservatives[165] and methanol from diet soft drinks.[114] The removal of ethyl mercury from vaccines and the reduction of aspartame consumption have removed these environmental factors from prime suspicion for inducers of autism (see discussion above), despite the publication of hypothetical mechanistic underpinnings. Thus, the availability of a hypothetical mechanism for the induction of autism by a particular agent is not a good indicator for its actual role in the pathogenesis of disease. However, given the weight of the burden of autism on society, it seems reasonable to test all plausible environmental triggers for the induction of autism.

Albeit indirectly, yet another factor points toward a role of acetaminophen in the development of autism: the lack of any published association between cystic fibrosis (CF) and autism stands out as the exception to the rule of association between inflammatory conditions and autism. CF is clearly associated with mucosal inflammation[166] but no connection between autism and CF has been published, suggesting that if any association does exist, it is not as conspicuous as that seen with other inflammation-associated conditions (e.g. cerebral palsy, preterm birth, or Down syndrome). Indeed, physicians at two independent clinics, each having treated hundreds of CF patients, recalled no patients with autism whatsoever in discussions with one of the authors (WP). An explanation for this potential exception to the rule of association between inflammation and autism lies in the fact that adults and children with CF tend to metabolize acetaminophen through phase II pathways much more so than do healthy controls.[167,168] This feature of metabolism in CF patients may be a "consequence of disease-specific changes in both enzyme activity and/or drug transport within the liver."[168] In this regard, CF patients are very different from patients with autism, who tend to have impaired phase II metabolism (Figure 3). Although speculative at present, the idea that CF may be protective from autism merits further study and may provide insight into the pathogenesis of the latter.

Why evaluate the connection between post-partum acetaminophen exposure and autism?

The possible role of acetaminophen exposure in neonates and young children in the pathogenesis of autism demands further study for a number of reasons. First, as pointed out above, the odds ratio for autism associated with acetaminophen exposure in children is one of the highest ever reported, exceeding the odds ratios consistently reported for exposure in utero. The initial study based on surveys[156] was conducted almost 10 years ago and has yet to be confirmed or refuted by more rigorous studies.

Perhaps even more compelling than the reason above, a second line of evidence

points toward the need to evaluate the connection between post-partum use of acetaminophen and autism. This evidence is based in part on two important epidemiological observations made by Bernard Rimland, founder of the Autism Research Institute in 1967 and the individual responsible for undermining the horrendous "refrigerator mother" hypothesis that prevailed during the first 20 years of research on autism. As shown in Figure 2, Rimland noted a rapid rise in the rate of autism beginning in the early 1980s.[169] Although he did not connect this rise with the use of acetaminophen in neonates and children, the widespread use of acetaminophen in infants, neonates, toddlers, and small children began in the early 1980s as a result of the discovery that aspirin may be associated with Reye syndrome.[170] This apparent rise in the prevalence of autism starting in the early 1980s has been corroborated by data collected in the state of California (Figure 2, lower diagram). In addition to an increase in prevalence, Rimland also noted a simultaneous increase in the relative ratio of regressive to infantile autism (Figure 2), as might be expected if a new and extremely potent trigger for the disease was introduced into the population which affected newborns and small children but not necessarily fetuses.

Rimland's study was not the last to suggest the emergence of a preponderance of regressive autism. In 2010, Ozonoff and colleagues published a prospective evaluation of behavior in children who would eventually be diagnosed with autism.[171] Ozonoff collected data starting at 6 months of age and focused on at-risk children, mostly those with siblings that had autism. Surprisingly, more than 85% of the children eventually diagnosed with autism were indistinguishable from neurotypically developing children at 6 months of age, but showed declines in social communication between 6 and 18 months. Thus, shortly prior to 2010, when Ozonoff's data were collected, most children with autism apparently presented with a regressive phenotype.

Intuitively, a preponderance of the regressive phenotype weakens any assumption that prenatal exposure is centrally or exclusively important. Indeed, there is no "proof" that autism is based on architectural changes laid down before birth. Associations between imaging and neuropathic findings and phenotype have been found,[172,173] but no one has systematically assessed the extent to which documented brain architectural differences in individuals diagnosed with autism (a diagnosis that cannot presently be made prenatally or in the first year and a half of life) derive from altered prenatal neurodevelopmental processes as compared with tissue changes acquired over time from disturbances in key phenomena such as excitation/inhibition balance, bioenergetics, immune function, or other metabolic processes. Indeed, it is widely appreciated that pathophysiological changes can be set off not just by early genetic or environmental influences but also in many ways at many times of life.

Another compelling reason to probe the post-partum acetaminophen–autism connection is that, if indeed acetaminophen exposure during early childhood is found to be an important player in the pathogenesis of autism, then reduction of the incidence of autism is readily achievable, with the primary concern being establishing best practices for the treatment of fevers and pain in children under 6 months of age. Given that acetaminophen exposure in pregnant women is associated with an increase in the risk of autism,[124] it would be quite surprising and perhaps very informative from a mechanistic perspective if indeed a dramatic reduction in acetaminophen exposure during early childhood did *not* cause a dramatic reduction in the incidence of autism.

Yet another reason to investigate the role of early childhood exposure to

acetaminophen in the pathogenesis of autism is that eliminating acetaminophen exposure early in life is unlikely to cause net harm, regardless of the effect on autism. Acetaminophen has never been shown to save lives in any controlled study and, as described above, is known to be associated with the induction of asthma and the presence of a variety of developmental delays. Going even further, Ohlsson and Shah state in their recent Cochrane report of acetaminophen use during a major thoracic surgery frequently performed on infants (surgical closure of the patent ductus arteriosus):

> *In view of a recent report in mice of adverse effects on the developing brain from paracetamol [acetaminophen], and another report of an association between prenatal paracetamol and the development of autism or autism spectrum disorder in childhood, long-term follow-up to at least 18 to 24 months postnatal age must be incorporated in any studies of paracetamol in the newborn population. Such trials are required before any recommendations for the use of paracetamol in the newborn population can be made.*[174]

In other words, Ohlsson and Shah argue that acetaminophen should *not* be recommended, even for major and necessary surgical procedures, until it is known whether or not it does in fact cause autism.

Another reason to conduct a study of the early childhood acetaminophen–autism connection is the observations of parents. During the 70-year history of investigating the pathogenesis of autism, scientists and pediatricians have made two well-documented and costly errors, both associated with dismissing the observations of parents. The first error was the acceptance for 20 years of the ''refrigerator mother'' hypothesis.[175] The second error was a firm belief, held until 2005, that autism could not be regressive and associated with a decline in previously existing neuropsychiatric function. At present, half of all parents of children with autism suspect vaccines as an underlying cause of their child's condition.[176] One study found that 29% of all mothers, with or without a child with autism, believe that autism can be induced by vaccines.[177] Other studies,[178] as well as surveys by various polling organizations such as Harris, Thompson Reuters, and the National League of Consumers, have reached similar conclusions. For reasons discussed above, it seems apparent that vaccines are not the underlying cause of the epidemic of autism, but at the same time, if history is any indication, ignoring the perspectives of parents is a grave error. The idea that vaccines induce autism has been widely blamed[179] on a single article published by Wakefield,[180] but articles published in scientific journals are unlikely to sway public opinion. Even repeated publications regarding the dangers of acetaminophen for neural development, for example, have had little impact on the use of acetaminophen in the pediatric population. Rather, it is social networks that are more likely to be persuasive. Acceptance of social networks is deeply ingrained in the human psyche,[181] most likely because of their ancient and critical importance for human survival.[182,183] Thus, parents of autistic children are likely to believe that vaccines are responsible for the induction of autism not because of a peer-reviewed article but rather because of their observations and the observations of individuals within their trusted social networks. With this in mind, the story of Steve Schultz is informative. He was convinced that his son's autism was induced by a vaccine because of what was obvious to him as an observer. Thus, Schultz's work both accounts for parents' observations and for the science surrounding the pathogenesis of autism. Whatever the factor inducing the development of autism, it is critical that the

scientific community learns from its past errors in ignoring the observations of parents.

It is estimated that the lifetime cost of supporting an individual with autism is currently US$2.4 million for an individual that also has an intellectual disability, or US$1.4 million for an individual with autism but without an intellectual disability.[184] It is also estimated that 38% of individuals with autism in the United States also have an intellectual disability.[185] This leads to an overall average cost estimation of approximately US$1.8 million to support one child with autism for his or her lifetime. We estimate that the total cost of running a definitive clinical study to test the connection between postnatal acetaminophen exposure and the induction of autism is less than the cost of caring for three to five individuals who have already developed autism.

Conducting an "acetaminophen withdrawal study": Practical considerations

Given the already established adverse effects of acetaminophen on the developing brain, described above, and the known risk of asthma following acetaminophen exposure, it seems inappropriate to intentionally expose anyone under the age of 5 years to acetaminophen for the purpose of a medical study. Rather, other approaches must be considered. One approach involves large-scale experiments in which acetaminophen exposure is essentially eliminated until the age at which a patient can be considered to be at zero risk for regression into autism. Such an "acetaminophen withdrawal study" would not be trivial. The feasibility of an acetaminophen withdrawal study was previously examined for the purpose of evaluating the connection between acetaminophen and asthma.[186] However, that feasibility study involved only 120 infants admitted to hospital after birth, at a time when some acetaminophen exposure may have already happened (e.g. during circumcision). Further, the study allowed some use of acetaminophen in all patients, and only involved 3 months of restricted use in the experimental population versus liberal use in a control population. Complete withdrawal up until the age of 5 years, on the other hand, would require all forms (injectable, oral, and suppository) of acetaminophen to be prospectively identified and reduced to an "essential minimum," if not eliminated. A few thousand patients may need to be enrolled, depending of course on the magnitude of the anticipated effect. However, fewer patients may be needed if at-risk populations (e.g. preterm delivery, Down syndrome) are selected for the study. The practices associated with circumcision will need to be reconsidered, and the use of acetaminophen during vaccinations, a common but already highly questioned practice,[187,188] would need to be eliminated. The possibility of passing acetaminophen to nursing infants and children through breast milk would also need to be eliminated. Extensive education of parents and a wide range of health-care workers (pharmacists and all physicians and nurses associated with obstetrics and pediatrics) regarding the avoidance of acetaminophen would be necessary. Further, comparable control groups with liberal use (current standard) of acetaminophen would need to be monitored simultaneously in order to obtain unequivocal results. Unfortunately, follow-up would require some time, and it would take years before a result is obtained. However, it is hoped that the weight of the current evidence will be considered sufficient for a dramatic reduction in acetaminophen use until incisive studies are completed.

A primary concern with an acetaminophen-elimination experiment would be the treatment of fever and pain. Alternative pharmacological methods of treating pain

are available. Although all pharmacological methods have drawbacks, acetaminophen is not considered to be highly effective for pain relief.[189] Indeed, as stated by McCullough, "The few clinical studies of (acetaminophen) and ibuprofen in children have struggled to find objective measures of pain capable of reliably distinguishing between active treatment and placebo."[189] Thus it seems unwise to risk potentially permanent neurological injury for apparently ineffective pain relief. Alternative pharmacological means are also available for fever reduction, but these have drawbacks. On the other hand, traditional methods of fever reduction (physical, non-pharmacological) look promising and have few side effects. However, these may be less convenient for clinicians and parents alike, and may prove ineffective in some cases. At the same time, the specter of inducing autism while treating pain or fever with acetaminophen should be weighed when considering the benefits of using acetaminophen, even in dire circumstances.

Given the current value attributed to the therapeutic effects of acetaminophen, it is worthwhile to consider possible ways to utilize the drug in the pediatric population, even if it is shown to be responsible for the epidemic of autism. One possibility is that individuals who are susceptible to acetaminophen-induced autism (e.g. perhaps those whose mothers have excessively high levels of vitamin B) might be identified, and use of the drug in these individuals could be avoided while others could benefit from use of the drug. Another possibility is that co-administration of N-acetyl cysteine (NAC) with acetaminophen could be used to attenuate the toxic effects of acetaminophen in cases where the use of acetaminophen is considered highly desirable. It might be hypothesized that any insufficiencies in phase I or phase II metabolism (Figure 3), which may lead to acetaminophen-induced autism, would be prevented by co-administration of NAC. NAC works both orally and parenterally, which may be important since acetaminophen is available for oral, intravenous, or rectal administration (Table 3). In patients with normal liver and renal function, the half-life of NAC is slightly longer than the half-life of acetaminophen, so a single dose of NAC may be sufficient to cover one dose of acetaminophen. However, the respective half-lives of the two compounds would need to be evaluated in the treatment population, and the idea that NAC may be protective against acetaminophen-induced autism is speculative.

Education of medical professionals is imperative for any acetaminophen withdrawal study, given the current medical environment. Acetaminophen use is currently ubiquitous and thought to be the only humane approach to pain and fever reduction for children from the time of birth to 6 months. The drug is available in a very wide range of formats for both prescription and over-the-counter use (Table 3). Almost one-quarter of all infants are given acetaminophen in any given week when in the hospital, making it the number one medication used in infants.[190] Given that most medical professionals currently practicing began their careers after 1982, it is not surprising that acetaminophen use is deeply engrained in practice and that alternatives may seem foreign and even primitive. Further, the idea that acetaminophen exposure in early childhood could be dangerous is extremely novel and even disturbing to most medical practitioners (personal observation by co-author WP). Even the best-trained pediatricians are generally unaware that the long-term effects of acetaminophen were never tested in children in controlled trials.[191] Further, with the exception of toxic metabolite production (Figure 3) that primarily affects liver function in adults but not babies, most physicians are largely unaware of the varied and complex

Table 3. Available US acetaminophen products from birth to early childhood.

Ingredient	Dosage Form	Strength	Indication	Special Considerations
Injectable				
Acetaminophen	Solution	1000 mg/100mL	Arthralgia; dental pain; fever; headache; mild/moderate/severe pain; musculoskeletal pain; myalgia; patent ductus arteriosus treatment[a]; post-operative/procedural pain[a]	Not studied in infants <28 weeks gestational age
Oral				
Acetaminophen (natural berry, grape, white grape, cherry, and bubble gum flavors)	Solution	120 mg/5 mL 160 mg/5 mL	Arthralgia; dental pain; fever; headache; mild/moderate/severe pain; musculoskeletal pain; myalgia; post-operative/procedural pain	All dosage forms available OTC
	Liquid	160 mg/5 mL 500 mg/15 mL		
	Suspension	160 mg/5 mL 325 mg/10.15 mL 80 mg/2.5 mL 80 mg/0.8 mL		
	Drops	80 mg/0.8 mL		
	Chewable tablet	80 mg 160 mg		
	Rapid tablet	80 mg 160 mg		
Acetaminophen/ Codeine[b] phosphate	Solution	120 mg/5 mL	Mild/moderate pain; Arthralgia[a]; bone pain[a]; cough[a]; headache[a]; myalgia[a]	Not recommended: for use in neonates, for post-operative tonsillectomy and/or adenoidectomy pain management; contraindicated in patients who are CYP2D6 ultra-rapid metabolizers

(continued)

Table 3. Continued.

Ingredient	Dosage Form	Strength	Indication	Special Considerations
Hydrocodone bitartrate[b]/ acetaminophen	Solution	108 mg/5 mL 167 mg/5 mL 163 mg/7.5 mL 217 mg/10 mL	Cough[a]; moderate/severe pain; post-operative/procedural pain[a]	Not recommended in children <2 years of age
Oxycodone hydrochloride[b]/ acetaminophen	Solution	325 mg/5 mL	Moderate/severe pain; post-operative/procedural pain[a]	
Acetaminophen/ chlorpheniramine[b]/ dextromethorphan[b]	Suspension OTC	7.5 mg/5 mL	Common cold; cough	Not recommended in children <4 years of age
Acetaminophen/ dextromethorphan[b]	Suspension	160 mg/5 mL	Cough; sore throat	Not recommended in children <4 years of age
Acetaminophen/ chlorpheniramine[b]/ dextromethorphan[b]/ Phenylephrine[b]	Effervescent tablet Suspension	250 mg 160 mg	Common cold; cough; fever; headache; mild pain; nasal congestion; pharyngitis; rhinorrhea; sneezing	Both dosage forms available OTC; not recommended in children <4 years of age
Suppository				
Acetaminophen	Rectal suppository OTC	80 mg 120 mg 325 mg	Arthralgia; dental pain; fever; headache; mild/moderate/severe pain; musculoskeletal pain; myalgia; post-operative/procedural pain	Not studied in infants <28 weeks gestational age

"Over-the-counter" is abbreviated OTC.
[a]Off-abel
[b]Combination products contain varying amounts of other active ingredients. The strength listed is for the acetaminophen component only.

effects of acetaminophen on the nervous system and on metabolism. Few practitioners are aware of the well-established connection between asthma and acetaminophen use, and fewer still are aware of the increased possibility of cryptorchidism (undescended testis) known to be associated with acetaminophen use.[192]

The exceedingly common and widespread use of acetaminophen both in the hospital and at home may have led to some degree of complacency in proper dose administration. For example, in a study of outpatient care prescription errors, 15% of children who were prescribed acetaminophen were given a dosage by medical professionals that was higher than recommended.[193] Similarly, another study found that 12% of children who were prescribed acetaminophen at a pediatric emergency department were given a prescription for acetaminophen higher than the recommended dosage.[194]

Interestingly, Li et al.[195] found that parents administered acetaminophen in a manner comparable to physicians; dosages administered by parents exceeded recommended amounts about 15% of the time. Li et al.[195] also noted that infants were more likely than older children to be given an inaccurate dosage of acetaminophen ($RR = 1.40,\ P < 0.04,\ CI\ 95\% = 1.06–1.86$) by their parents. This latter observation is particularly concerning since it is during infancy that the brain may be most sensitive to damage by acetaminophen from oxidative stress. However, it is unknown to what extent acetaminophen-induced neuropathology can be induced by the recommended dose of the drug, and to what extent excessive doses are responsible for the induction of neurological disorders in the pediatric population.

The bottom line is that hundreds of studies describing the epidemiology of autism and the numerous and varied risk factors for autism have a straightforward explanation: autism could be an acetaminophen-induced brain injury facilitated by oxidative stress and inflammation in newborns and young children. This is certainly an attractive view from an intellectual perspective, as it satisfies Occam's razor. Most importantly, this model merits urgent testing because (a) it is intuitive and accounts for the observations, (b) the experiment to test the hypothesis is very feasible, and (c) if proven correct, the prevalence of autism in future generations will be dramatically reduced. The urgency of the issue cannot be underestimated, as precious time and resources that could be allocated much more constructively and usefully in this time of serious need are being poured into approaches that offer little to no hope of prevention.

Acknowledgements

The authors are very grateful to Stephen M. Edelson, current director of the Autism Research Institute, for sharing his knowledge of the work with autism that he and Bernard Rimland performed during the 1960s through the 1990s. The study team is also very grateful to Susanne Meza-Keuthen for her generous financial support of this study. The authors wish to thank Michael R. Knowles, Jeffrey P. Baker, Margaret Leigh and Carolyn Durham for very helpful discussions.

Declaration of conflicting interest

The Authors declare that there is no conflict of interest.

Funding

This review received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval

No studies using animal models and no research involving human subjects were performed for this study.

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