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Autism Insights

Role of Environmental Exposure to Toxins and Microbial Infections in Autism

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ABSTRACT: Autism is a complex developmental neurological disorder causing impaired function and structure of brain development. According to a recent report from the Centers for Disease Control and Prevention (CDC), autism is estimated to affect 1 in 88 children in USA.[1] In spite of several reports linking prenatal exposure to environmental toxins and to microbial agents via infections to a spectrum of autism and autism-like disorders, to date, neither the associated risk factor nor the pathophysiological mechanisms have been established unequivocally. The impact of these environmental agents is believed to be similar to that of other neuropsychiatric disorders. Earlier, we have reported the impact and immunological implications of mercury and viral infections in autism. In this review, we highlight the current incidence of autism, discuss brain development in autism, present the prominent features of neuroanatomy in autism, describe neurodegenerative findings in autistic individuals, summarize the hypotheses to explain autism, and provide a perspective of the molecular events in autism and autism spectrum disorders (ASD). The early events that trigger this complex cluster of neurological disorders may involve the breach of cellular interface, which leads to the influx of water which in turn damages the developing neurons during the early stages of brain development. Alternatively, neurodegenerative disorders can be caused by the interaction of environmental agents like heavy metals with transport proteins like aquaporins and gap junction protein complexes embedded in the neuronal network during synaptogenesis.

KEYWORDS: environmental chemicals, toxins, microbial infections, autism

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Introduction

Neurodegenerative diseases are considered as a group of disorders that seriously and progressively impair the functions of the nervous system through selective neuronal vulnerability of specific brain regions like the cerebellum and in particular the Purkinje cells.[2] Microbial infections in synergy with environmental toxicants like heavy metals may trigger processes that are directly or indirectly linked to degenerative disorders. In the event of a severe environmental insult, incomplete maturation of areas of the brain undergoing organization and development at the time of the insult may result in incomplete development. These events at the submolecular level involve the tight junction proteins (TJPs) of the epithelial cells from the nasopharynx, gastrointestinal (GI) tract, or endothelial cells of the blood–brain barrier (BBB) along with the gap junction proteins (GJPs) of the immune cells thus forming a quad comprising of the nasopharynx/gut/brain/immune axis concomitantly with other small solute transport proteins including aquaporins (AQPs). Previously, we had described how autoimmunity and viral infection in the presence of environmental heavy metal exposure aid in inflammation which may be the immunological trigger for autism.[3] In this review, we highlight the current incidence of autism, discuss brain development in autism, present the prominent features of neuroanatomy in autism, describe neurodegenerative findings in autistic individuals, summarize the hypotheses to explain autism, and provide a perspective on key molecular events (Fig. 1).

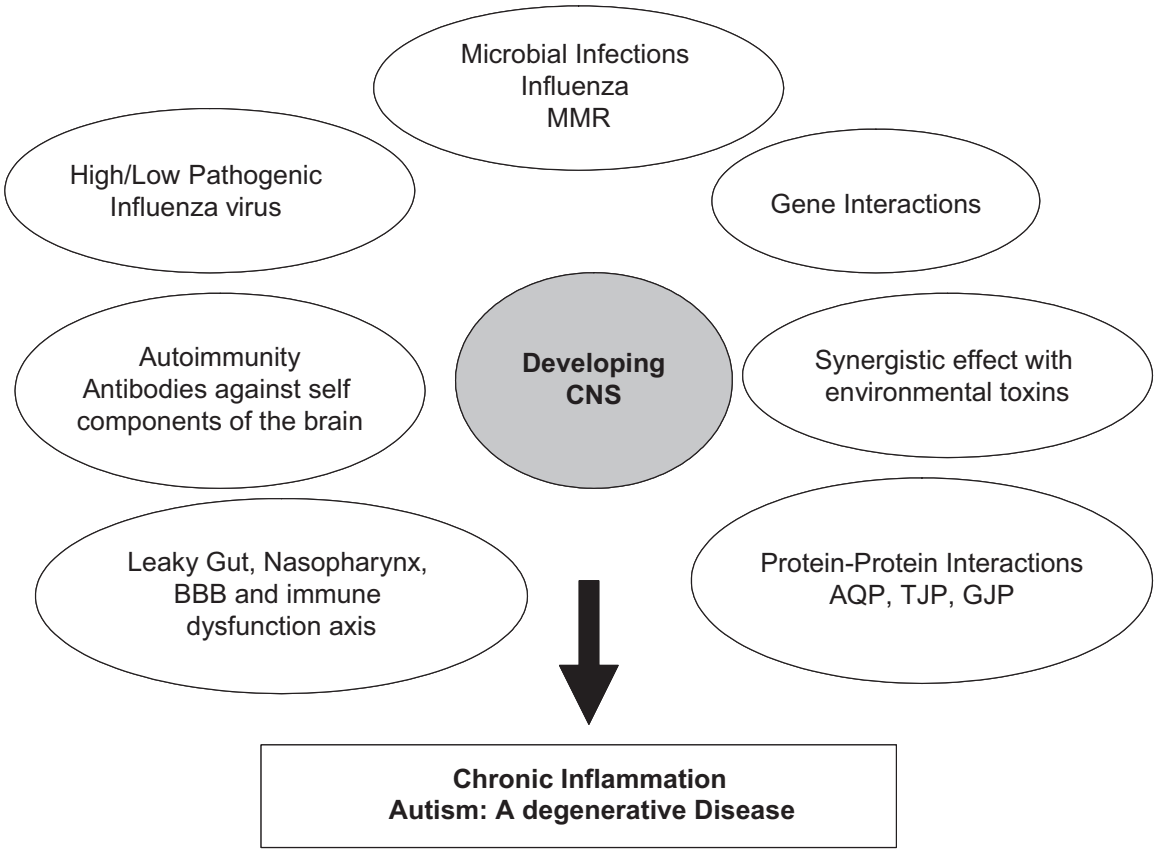

Figure 1. Autism: a neurodegenerative disease with environmental and microbial interactions.

Autism: An Urgent Public Health Issue

Autism was first described by Leo Kanner in 1943 as "children locked within themselves." Developmental disorders such as autism and autism spectrum disorders (ASD) encompass a large set of associated cognitive and neurobehavioral disorders, including impaired verbal and/or nonverbal communication. These spectrum of disorders may result in impaired socialization.[4] Autism affects 400,000 Americans, with 1–2 new cases per 1,000 births in the United States. Based on U.S. Census domestic and worldwide data, it is estimated that the number of people affected by the disorder is growing at a rate of roughly 1.2% annually in the U.S. and 1.4% annually worldwide.[5] It has been characterized as "a disorder of neuronal organization, that is, the development of the dendritic tree, synaptogenesis, and the development of the complex connectivity within and between brain regions."[6] Unlike other diseases or disorders, autism is undetectable other than by the behavior and communication of the affected individual. Autism is found in every country and area of the world and is present in all racial, ethnic, religious, and socioeconomic backgrounds.

Autism cannot be identified biologically with a simple medical test like a brain scan or a blood test. Autism in early childhood development is often identified by the abnormal functioning of learning, motor, and communication skills in children.[7] Many children spend their first years of life undiagnosed and untreated before the disorder becomes diagnosed by a physician. Autism has spread throughout the world at an increasingly alarming rate. The Autism Society of America considers autism to be an epidemic, and Dr. Jose Cordero, Director of the Centers for Disease Control's National Center on Birth Defects and Development Disabilities, confirms statistics that establish autism as an "urgent public health issue."[7] Epidemiological studies have revealed the importance of external and/or environmental factors and the modification in the factors. In 1994, ASD occurred once in 10,000 births, but as of today one child out of 166 births will have ASD. Of the many children who are born with ASD, five times as many males will be identified with having ASD than females. Reported statistics show that ASD is found in 1 in 54 males and 1 in 252 females.[7] Although a large proportion of individuals with autism manifest abnormal development from birth, a subset of at least 20–30% experience a regression with onset between 18 and 24 months of age after a period of apparently normal development.[8] Autism is commonly manifested by age 3 and characterized by impairments in social interaction and communication, as well as restricted, repetitive, stereotyped patterns of behavior. The neuropathologic findings and its characteristic behaviors are clinical manifestations of both pre- and postnatal alterations.

Brain Development in Autism

The brain of a fetus develops throughout pregnancy, and it is generally accepted that autism is caused by abnormalities in brain structures or functions during this stage. A very high proportion of cells of the nervous system are developed before birth. In normal development, cells migrate to a specific area of the brain and differentiate to take on special functions. Each neuron connects with other neurons, and in this way, communications are established between various areas of the brain and between the brain and the rest of the body. As each neuron receives a neuronal signal, it passes the signal to the next neuron. By birth, the brain has compartmentalization of different functions in distinct regions and subregions, but brain development does not stop at birth. The brain continues to change during the first few years of life, as new neurotransmitters become activated and additional lines of communication are established. Problems appear when normal brain development is impaired. Improper cellular migration in the brain may cause problems with the neural pathways or the neurotransmitters interfere with the coordination of sensory information, thoughts, feelings, and actions.

At the molecular level, depressed expression of neural cell adhesion molecules that are critical during brain development for proper synaptic structuring has been associated with autism.[9] Reductions in Purkinje cells, the largest neuronal cells found in the cerebellum, have also been reported in people with autism. Specifically, reduced Purkinje cell gamma aminobutyric acid (GABA) input to the cerebellar nuclei potentially disrupts cerebellar output to higher association cortices affecting motor and/or cognitive function.[10] These alterations in the GABAergic system contribute to a more widespread pathophysiology in autistic brains.

Many findings suggest a consistent association of unfavorable events in pregnancy, delivery, and the neonatal phase and the pervasive developmental disorders. The disabilities in autism emerge during the first three postnatal years and continue to evolve with ongoing development. In autism subtle changes in morphology of specific neurons, brain connections, and brain size occur.[11] The different regions that are possibly affected are the hippocampus, amygdala, frontal lobes, parietal lobes, cerebellum, and corpus callosum. Amygdala plays a significant role in emotional and social processing, particularly in the areas of cognition in which individuals with autism tend to perform much differently than typical controls. An experimental observation in a magnetic resonance imaging (MRI) study showed increased cortical thickness in the brains of children with autism.[12] Postmortem analyses on autistic and typically developing children revealed significantly increased cortical thickness in the autism group. This indirectly supports the observation that some children with autism have increased brain volumes and head circumference. Further, patients with autism show less amygdala activation when looking at faces than do "typically developing" individuals. There is greater left amygdala and left orbito-frontal cortex inactivation suggestive of social impairment. There is greater activation in the right anterior cingulate cortex and bilateral superior temporal sulcus in the autistic group than in controls.[13]

In postmortem brains, amygdalae of people with autism had significantly fewer neurons than controls, particularly in the "lateral nucleus." The lower neuron levels could be because of reduced production of neurons in the amygdala during early development or excessive neurodegeneration. It is possible that autism could be a result of degeneration of amygdala and possibly Purkinje cells. Autism is proposed to be associated with abnormalities of information integration that are caused by a reduction in the connectivity between specialized local neural networks in the brain and possible overconnectivity within the isolated individual neural assemblies.[14] Furthermore, abnormal connections between brain areas might explain some of the symptoms of autism. Perceptual abnormalities in autism are associated with alteration of induced gamma activity patterns overlying visual cortical regions. Autistic participants showed abnormal gamma activity to reflect decreased "signal to noise" because of decreased inhibitory processing.[15] Regional brain volume results were found to be variable and inconsistent. Thus, no one "autism region" was found to be consistently affected using neuroimaging techniques. Neuroimaging has identified a social brain network and shown that the social brain does not function normally in people with autism. Neuroimaging has also begun to explain why people with autism may have superior skills in some domains.[16]

Proper formation of connections in the brain during childhood provides the substrate for adult perception, learning, memory, and cognition. Improper formation or function of these connections leads to many neurodevelopmental disorders, including autism. Autism can also be caused or influenced by nongenetic factors. Specifically, maternal viral infection has been identified as the principal nongenetic cause of autism. Recent studies have even indicated a genetic link between autism and immune system genes.[17] Since immune molecules are increased following infection and are present in the developing brain, it is possible that changes in these immune molecules lead to changes in neuronal connectivity that underlie some forms of autism. Furthermore, shape abnormalities in the basal ganglia have been shown to be a good predictor of motor, social, and communicative deficits in boys with ASD.[18] Genetic studies suggest a significant association of malformations of coevolved subcortical structures along with the basal ganglia[19–21] and similar degeneration can be expected in ASD.

Neuroanatomy of Autism

Cells of the cerebral cortex are arranged into functional units called minicolumns.[22] Each of these tiny vertical structures is made up of about 100 neurons that act as a cohesive unit. The axons and dendrites, which neurons use to send and receive information, form fiber bundles that connect the columns to one another. There is a significant difference between brains of

autistic patients and controls in the number of minicolumns, in the horizontal spacing that separates cell columns, and in their internal structure, that is, relative dispersion of cells. In autism, there are minicolumnar abnormalities in the frontal and temporal lobes of the brain. Specifically, cell columns in brains of autistic patients were more numerous, smaller, and less compact in their cellular configuration with reduced neuropil space in the periphery.[23] The presence of narrower minicolumns supports the theory that there is an abnormal increase in the number of ontogenetic column units produced in some regions of the autistic brain during corticoneurogenesis.[22]

One prominent feature in the neuroanatomy of autism is the tendency to have unusually large brains.[24] Brains affected by autism have a growth spurt shortly after birth and then slow in growth a few short years afterward. The younger but not older brains are larger in autism than in controls. The white matter contributes disproportionately to this volume increase and in a nonuniform pattern suggesting postnatal pathology, that functional connectivity among regions of autistic brains is diminished, and that neuroinflammation (including microgliosis and astrogliosis) appears to be present in autistic brain tissue from childhood through adulthood.

The most consistent pathological abnormality found in autopsied cases of individuals with ASD is a decrease in the number of cerebellar Purkinje neurons.[25] Purkinje cells in the cerebellum are rich in the neurotransmitter serotonin, which is responsible for inhibition. Interestingly, abnormal levels of serotonin are well-documented in individuals with autism and may be linked to problems in mood regulation. Purkinje neurons undergo cell death in circumstances that may cause debilitating damage in other brain areas.

Purkinje cell vulnerability is associated with the following 10 causes: (1) ischemia,[26] (2) hypoxia,[26,27] (3) excitotoxicity,[28–32] (4) G protein dysfunction,[33,34] (5) viral infections,[35] (6) vitamin deficiencies,[28] (7) heavy metals,[36–42] (8) toxins,[30,31,43–46] (9) chronic malabsorption syndrome,[47–49] and (10) oxidative stress.[50–53] It's conceivable that a cluster of events acting in synergy can play a role in increasing the risk of autism and ASD.

Neurodegenerative Findings in Autism

A biological basis of autism was suggested by the finding of developmental hypoplasia in lobules VI and VII of the cerebellar vermis.[54] The ontogenetically, developmentally, and anatomically distinct vermal lobules I to V were found to be of normal size. However, the relationship of cerebellar vermal atrophy to infantile autism has been disputed.[55] They found that the average relative size of lobules VI and VII of the cerebellar vermis was no different in their 13 patients with infantile autism when compared to that of 125 normal individuals. They found relative hypoplasia of lobules VI and VII in patients with Rett syndrome and Sotos cerebral gigantism, which are two disorders characterized by autistic behavior. No relative vermian atrophy was seen in other disorders associated with autistic behavior: fragile X, Angelman, adult phenylketonuria, and Sanfilippo. Furthermore, they found a relative atrophy of lobules VI and VII in several patients with primary cerebellar hypoplasia and Usher syndrome type II, syndromes not associated with autistic behavior. Autopsy and neuroimaging studies have suggested that ASD is caused in part by abnormal brain development. The central nervous system (CNS) structure most consistently affected in individuals with autism is the cerebellum, with a decrease in the number of Purkinje cells, which are present in the majority.[56–58] Neurodegenerative signs are for the most part absent from these autopsy samples, suggesting a developmental defect. Neuroimaging studies have consistently demonstrated posterior cerebellar hypoplasia. Although the cerebellum has classically been considered a motor control center, functional imaging studies indicated that the cerebellum is also active during cognitive tasks that are defective in ASD, including language and attention. Thus, the identified cerebellar defects may contribute directly to some of the behavioral abnormalities associated with ASD. In turn, genetic alterations that perturb cerebellar development may contribute to ASD susceptibility.

Emerging Hypotheses

The changes in Purkinje cell physiology and its vulnerability to environmental stresses can be equated to the postnatal cell loss. The other phenomenon which has to be accounted for is the increased brain volume in autism concomitant to the Purkinje cell loss. Inherent in this equation is the association of toxicity caused by infection or heavy metals, which may elicit neuronal insult caused by oxidative stress and thus coupling the possible involvement of glutathione. Studies have shown that decreased glutathione levels and increased oxidative stress may play a role in the pathology.[57,58] Typically, high concentrations of sulfhydryl-reactive metal cations: mercury, lead, bismuth, cadmium, and arsenic are found in autistic children.[59–65]

Cerebellum Size and Abundance of Purkinje Cells

The size of the cerebellum is reported to be smaller in individuals with autism, and the number of cells that direct messages to other brain areas is less. Further, males are at more risk of neuropsychological diseases associated with cerebellar pathology, and in animal models there are noticeable sex differences in the response to insult and genetic mutation.[66] While the mechanism that affects Purkinje cell number and cerebellar size is not yet well described, preliminary data suggest that reelin, an autism candidate gene, interacts with gonadal sex hormones during cerebellar development. An interaction of abnormal reelin expression coupled with exposure to differing levels of testosterone during brain development may contribute to the reduced number of Purkinje cells in individuals with autism.[67]

Oxidative stress produces Purkinje cell death and reduction in numbers. Importantly, research has found that

administration of an antioxidant protects Purkinje cells against oxidative stress. It is conceivable that some of these children become autistic from neuronal cell death or brain damage sometime after birth as a result of insult.[68]

Inflammation in the Brain

Abnormalities associated with the immune system include derangement of antibody production, skewing of T cell subsets, aberrant cytokine profiles, and other impairments consistent with chronic inflammation and autoimmunity.[69] Compared with normal control brains, the brains of the people with ASD featured immune system activation and inflammation in the brain. This ongoing inflammatory process was present in different areas of the brain and produced by cells known as microglia, and astroglia, cytokines, and chemokines were elevated and found in abnormal patterns consistent with inflammation. These elevated cellular protein levels indicate that they are part of the "innate" immune system in the brain, and appear to be caused by immune abnormalities from inside the brain.[70] The findings in the brain tissue were corroborated by studies of cerebrospinal fluid obtained from six children with autism (ages 5 to 12 years), in which cytokines that promote inflammation were found to be elevated.

Prenatal Stressors

In general, if exposure to environmental toxicants occurs before or after organ development, it is less vulnerable to perturbation than if exposure occurs during the development. There is considerable prenatal development in humans and thus it is a very critical period. Further, the critical period of neurodevelopment is during the transplacental period in the third trimester.[68] A peak of prenatal stressors in autism is at 25–28 weeks gestation.[71]

A. Chemical exposure. Three well-established environmental risk factors for ASD are thalidomide, valproic acid, and misoprostol. Prenatal exposure to chemical teratogens including the antiseizure medications thalidomide and valproate is associated with increased incidences of ASD. Exposure to these agents is known to produce brainstem abnormalities, which are reflected in individuals with autism. While the mechanism by which these defects are caused by these chemicals is being explored, the findings do raise the possibility that it may be similar to that which contributes to autism neuropathology. The use of drugs to control epilepsy (such as valproic acid) during pregnancy has been suggested to increase the risk of ASD in offspring. Exposure of rats to valproic acid during neural tube closure on embryonic day 12.5 leads to a condition that mimics autism. These rats had significantly fewer Purkinje cells in the cerebellar vermis and a reduction in volume.[72] Infection before birth termed prenatal infection in mouse causes differential expression of genes in brains of mouse progeny.[73]

B. Viral exposure. Viral exposure on day 9 of BALB/c mice causes a permanent structural and functional change in the brains of mice that leads to autism.[73] The neonatal affected brains showed a differential regulation of genes in the neonatal brain homogenates particularly AQP4. These results show that prenatal human influenza viral infection on day 9 of pregnancy leads to alterations in a subset of genes in brains of exposed offspring, potentially leading to permanent changes in brain structure and function.

C. Prenatal ultrasonography exposure. Case-controlled study of prenatal ultrasonography exposure in children with delayed speech showed that the brains of the offspring were damaged consistent with that found in the brains of people with autism.[74]

D. Hyperthermia. An extensive review of literature on maternal hyperthermia in a range of mammals found that CNS defects appear to be the most common consequence of hyperthermia in all species, and cell death or delay in proliferation of neuroblasts (embryonic cells that develop into nerve cells) is believed to be one major explanation for these effects.[75]

Postnatal Microbial Infection

Autism occurs either before birth or many months after birth, following a period of apparently normal growth and development. The disorder has been proposed to result from disruption of postnatal or experience-dependent synaptic plasticity.[76] There are significantly fewer neurons in the autistic amygdala overall and in its lateral nucleus. In conjunction with the findings from previous MRI studies, the autistic amygdala appears to undergo an abnormal pattern of postnatal development that includes early enlargement and ultimately a reduced number of neurons.[77]

Postnatal infection can be carried by breast milk.[78] It has been shown that the tropism of the virus from non-neurotropic (nasal origin) to neurotropic origin can occur by the transfer of the influenza virus from the mother to the newborn/suckling offspring. This was shown in the case of influenza A H3N2 virus, which penetrates the cerebral capillaries by accumulating viral antigens and increasing brain vascular permeability causing progressive brain edema after intranasal infection of the female in the newborn/suckling mice.

Infection Caused by Microorganisms

Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. Maternal viral infection is known to increase the risk of autism in the offspring. In an animal model, respiratory infection of pregnant mice (both BALB/c and C57BL/6 strains) with the human influenza virus yields offspring that display highly abnormal behavioral responses as adults. As in autism, these offspring display deficits in prepulse inhibition (PPI) in the acoustic startle response. At least some of these behavioral changes likely are attributable to the maternal immune response itself. That is, maternal injection of the synthetic double-stranded RNA polyinosinic-polycytidylic acid causes a PPI deficit in the offspring in the absence of virus. Therefore, maternal viral

infection has a profound effect on the behavior of adult offspring, probably via an effect of the maternal immune response on the fetus.[79]

It has been postulated that a subacute, chronic tetanus infection of the intestinal tract is the underlying cause for symptoms of autism observed in some individuals. Extensive antibiotic use has been associated with autism. Colonization of opportunistic pathogens occurs after disruption of protective intestinal microbiota. The vagus nerve is capable of transporting tetanus neurotoxin (TeNT) and provides a route of ascent from the intestinal tract to the CNS. Once in the brain, TeNT disrupts the release of neurotransmitters by the proteolytic cleavage of synaptobrevin, a synaptic vesicle membrane protein. This inhibition of neurotransmitter release would explain a wide variety of behavioral deficits apparent in autism. Lab animals injected in the brain with TeNT have exhibited many of these behaviors.[80]

Animal models in which early viral infection results in behavioral changes later in life include the influenza virus model in pregnant mice and the Borna disease virus model in newborn Lewis rats.[35,67,73,79,81] Many studies over the years have presented evidence both for and against the association of autism with various viral infections. However, in light of recent worldwide outbreaks of avian influenza caused by low and high pathogenic influenza strains[82,83] as well as human cases including in pregnant women,[84] we provide a perspective on molecular events associated with maternal prenatal influenza virus and autism.

Tight Junctions of Nasopharynx, Gut, and BBB

Nasopharynx and the GI tract are the possible routes of entry for the influenza virus in the human body. This entry is only possible by the breach of the intact epithelium. The epithelium damage to the tight junctions linking epithelial cells causes increased permeability allowing virus particles to enter through the mucosal barrier of the gut and respiratory tract into the bloodstream. Influenza first infects the upper airway and the ciliated cells in the bronchus and bronchioli. The respiratory epithelium is especially equipped to defend from incoming pathogens by a layer of mucus (bronchus), ciliated cells (bronchus and bronchioli). The initial cell target of human influenza viruses in a cell culture model of the human airway epithelium is the nonciliated type, while in contrast, influenza viruses from avian species infect ciliated cells.[85,86] A postmortem study of tissues of a pregnant women in China who died after infection with influenza strain H5N1 revealed viral genomic sequences and antigens in type II epithelial cells of the lungs, ciliated and nonciliated epithelial cells of the trachea, T cells of the lymph node, neurons of the brain, and Hofbauer cells and cytotrophoblasts of the placenta.[84] Irrespective of the cell type involved, the breach of the nasal epithelium is the only mechanism by which the virus will enter the body. Thus, the breach will involve the TJPs and GJPs.

In a mouse model, human influenza virus strain H1N1 caused behavioral symptoms in progeny very akin to what is seen in autism.[73] This would imply that the virus enters the progeny of the mice that have been exposed to influenza virus after 12.5 days of conception. This entry again is possible only when the placental barrier is easily breached such that the developing embryo having a leaky barrier allows the virus to damage the CNS development.

Gastrointestinal symptoms, inflammation, and dysfunction are observed in children with autism. Mild to moderate degrees of inflammation were found in both the upper and lower intestinal tract. In addition, decreased sulfation capacity of the liver, pathologic intestinal permeability, increased secretory response to intravenous secretin injection, and decreased digestive enzyme activities were reported in many children with autism.[87,88]

The BBB is a very specialized barrier system of endothelial cells that separates the blood from the underlying brain cells, providing protection to brain cells and preserving brain homeostasis (stability). The BBB formed by brain microvascular endothelial cells regulates the passage of molecules and leukocytes in and out of the brain. The brain endothelium has a complex arrangement of tight junctions between the cells that restrict the passage of molecules. This physical barrier is further enhanced by interactions with glial processes that form end-feet and surround the brain microvessels. Oxidative stress is a major underlying cause of neurodegenerative and neuroinflammatory disorders and BBB injury associated with them. Furthermore, serum S100B, a marker of neurological damage, significantly correlated to autistic severity. Autistic children had significantly higher serum S100B protein levels (207 ± 53 pg/ml) than healthy controls (171 ± 35 pg/ml). Patients with severe autism had considerably higher serum S100B levels (222.5 ± 53 pg/ml). This increase did not correlate with the serum levels of anti-ribosomal P proteins.[88] This study highlights the significance of finding new mechanisms to explain the elevated serum S100B levels in autism.

Importance of Proteins at the BBB

BBB, which is formed by the tightly packed endothelial cells that line cerebral microvessels, has an important role in maintaining a precisely regulated microenvironment for reliable neuronal signaling. This higher density restricts passage of substances from the bloodstream much more than endothelial cells in capillaries elsewhere in the body. Astrocyte cells called astrocytic feet surround the endothelial cells of the BBB, providing biochemical support to those cells. The association of brain microvessels, astrocytes, and neurons forms functional "neurovascular units," where endothelial cells participate in the modular organization. Specific interactions between the brain endothelium, astrocytes, and neurons regulate BBB function.[89] The BBB prevents many bloodborne toxins, bacteria, and viruses from infecting the nerve cells of the brain.

Matrix metalloproteinase-9 (MMP-9) and tissue inhibitors of metalloproteinases 1 (TIMP-1) play important roles in the function of the BBB. These metalloproteinases were determined in influenza virus infection, and this outcome suggests that MMP-8 and TIMP-1 damage the BBB. This injury is brought about by influenza virus infectivity.[90] After infection with influenza A virus, the BBB was penetrated and destroyed. Thus, infection by influenza A virus increases brain vascular permeability.[91] If the permeability is altered in adults, the consequence of influenza virus infection in developing infants would be damaging to the neuronal cells of the brain. The infectivity and pathogenicity of influenza virus are primarily determined by host cellular trypsin-type processing proteases, which cleave the viral membrane fusion glycoprotein hemagglutinin (HA). Therefore, the distribution of the processing protease is a major determinant of the infectious organ tropism.[92]

Abnormal neuronal development was associated with vascular malformations and a leaky BBB. Leakiness causes increased uptake of fluorescent albumin by neurons, but not glia.[93] Abnormal neuronal development was associated with vascular malformations and a leaky BBB.[94] Protein extravasation and uptake of fluorescent albumin by neurons, but not glia, was commonly associated with abnormal cortical development. Neuronal hyperexcitability was also a hallmark of these abnormal cortical regions. These results suggest that prenatal vasculogenesis is required to support normal neuronal migration and maturation. Altering this process leads to failure of normal cerebrovascular development and may have a profound implication for CNS maturation.[93]

TJPs

TJPs regulate the paracellular pathway for the movement of ions and solutes in-between epithelial cells. TJPs consist of the transmembrane proteins occludin and claudin, and the cytoplasmic scaffolding proteins ZO-1, -2, and -3.[95] Tight junctions are the primary mechanism that regulates whether the epithelium is tight or leaky. This is because of proteins in the claudin family that form a seal to both restrict paracellular diffusion and permit specific transport of ions between cells across the epithelial barrier. There are nearly 20 different claudins, and cells simultaneously express several claudins. How cells use multiple claudins to regulate epithelial barrier function is unknown.

GJPs: Brain Neuronal Cells and Immune Cells Connection

GJPs consist of channels composed of proteins in the connexin family[96] and are important for migration of embryonic neurons to the cortex.[97] Gap junction channels enable the direct diffusion of molecules from one cell to its nearest neighbor. These molecules include metabolites (ATP), antioxidants (glutathione), signaling molecules (cAMP, inositol trisphosphate), and ions (calcium). The sites where certain neurons connect, called neuronal gap junctions or electrical synapse,[98] are sensitive to environmental agents and have downstream effects on cell growth and reproduction, and possibly on migration of neuronal precursors to their appropriate destinations. Disruption of the gap junction by biophysical and environmental factors will help identify the cause of cortical malformation. This will be relevant to a better understanding of brain development as well as the causes of ASD. Deficits in neuronal migration and formation of different layers of the cortex may lead to the neuropathological and behavioral features observed in autism.

Autism has a dominance of CD8+ cells, together with increased intraepithelial lymphocytes in the GI tract. Proliferation of foveolar epithelium was similarly increased in autism. These findings demonstrate a focal CD8-dominated gastritis in autistic children.[99] Further, TJPs/GJPs have been associated with intestinal intraepithelial cells, and thus a possibility to play a role in gut and immune connection and also an immune and brain connection.[100] Mice with a mutation in GJP in neuronal cells, called neuroligin-3, showed an increase in the signaling of inhibitory neurotransmitters and an impaired ability to interact socially with other mice.[101] This could serve as a good experimental animal model where there is social defect without a mental deficit in autism.

AQPs

The regional and global elevations in white matter T2 suggest abnormalities of white matter tissue water content in autism, which may represent a neurobiological basis for the aberrant cortical connectivity hypothesized to underlie the disorder.[102] The flux of water is controlled by a family of bidirectional water channel proteins called aquaporins (AQPs; Fig. 2). Transcripts of AQP1, AQP3, AQP4, AQP5, AQP8, and AQP9 are detected in the brain.[103] Especially in astrocytes, AQP4 is abundantly expressed in end-feet at the BBB. Brain AQPs play important roles in the regulation of water homeostasis and the cerebrospinal fluid formation. Recently, AQP4 and to a smaller extent AQP9 have been reported to be involved in the brain water accumulation in brain edema. Studies of transgenic mouse and brain injury models reveal that AQP4 may play a role not in the edema formation, but in the fluid elimination.[104] AQP4 mRNA is upregulated where the BBB is compromised.[105]

Prenatal human influenza viral infection on day 9 of pregnancy in BALB/c mice leads to alterations in a subset of genes in brains of exposed offspring leading to autism.[73] This gene regulation was determined in offspring of BALB/c mice using microarray technology. The results showed that autism leads to permanent changes in brain structure and function, especially dealing with transporters in particular AQP4. The nonfunctional aquaporin AQP11 is expressed in Purkinje cell dendrites, hippocampal neurons of CA1 and CA2, and cerebral cortical neurons.[106] Purkinje cells are target cells for autism. The defect in AQP11, addition or some change in the gene sequence or some peptide signaling, may alter the

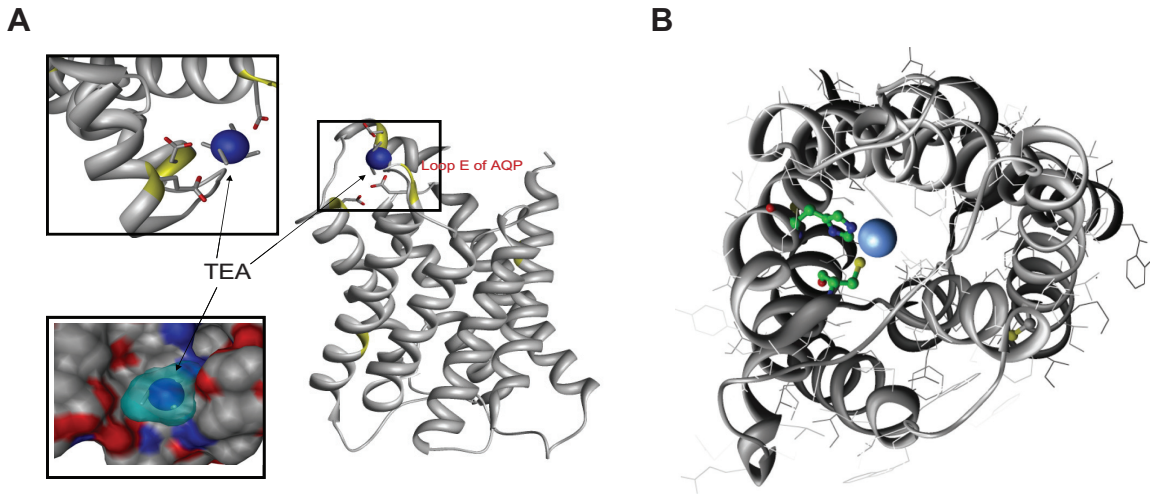

Figure 2. (**A**) Interactions of trialkylammonium cation with loop E of AQP1 at the heavy metal interaction site. (**B**) In silico model of Hg^{2+}–AQP1 complex indicative of pore constriction.

function of branching in dendrites. AQP11 has a tri-cysteine motif, a high affinity mercury ion (Hg^{2+}) binding site. However a specific role for this binding event in a nonfunctional AQP is yet to be identified. Most AQPs have the potential to interact with cations including copper (Cu^{2+}), mercury (Hg^{2+}), and cadmium (Cd^{2+}) at the triethylammonium cation binding site as illustrated in Figure 2 which encompasses the loop E.[107] These interactions could potentially alter pore geometry and alter solute transport (Fig. 2B). Furthermore, environmental exposure to various endocrine disrupting chemicals (Fig. 3) and parasitic infections[108] can simultaneously alter the expression of multiple targets (AQP, TJP, GJP) indicating the importance of studies focused on the role of environmental factors altering barrier proteins and transport to get a clear understanding of risk factors pertinent to ASD.

Genetics of Autism

Although autism has been associated with many genetic and environmental causes, it is not a single disorder, but rather a complex disorder that has distinct causes but they often

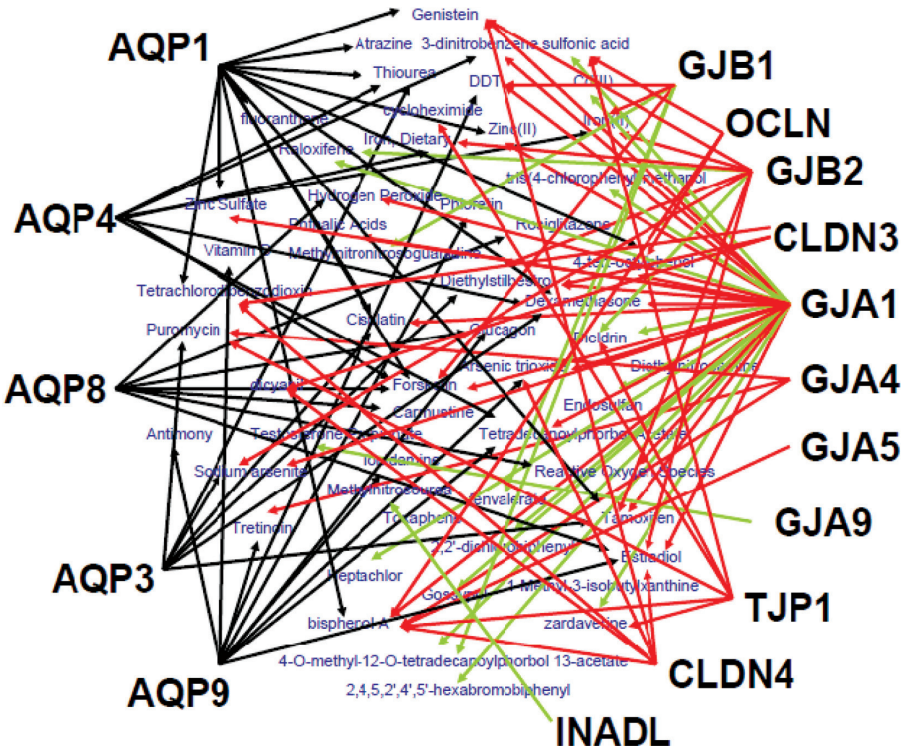

Figure 3. Environmental exposure to chemicals that affect multiple targets implicated in ASD (AQPs [LHS], barrier/junction proteins [RHS]).

co-occur. An estimated heritability of above 90% was reported in early studies of twins. The risk of having one or more features of the broader autism phenotype might be as high as 30% for adult siblings illustrating the importance of genetics. Chromosomal abnormalities including deletion, duplication, and inversion are implicated in autism. Multiple mutations and involvement of more than one gene are often implicated. Furthermore, different sets of mutations may be involved in different cases. To date, the actual mutations or sets that increase the risk of autism have not been identified. The ideogram generated based on reported studies suggests that every human chromosome may be involved.

Using array comparative genomic hybridization, spontaneous alterations in the genetic material have been reported for about 10% of families with one autistic child. The study also illustrated that a substantial fraction of autism may be highly heritable but not inherited. Exposure to known teratogens such as thalidomide, valproic acid, or misoprostol during early weeks of pregnancy, though rare, is related to the risk of autism.

Interactions among related genes revealed that complex genetic interactions account for autism risk. The genes involved normally in the dampening of nerve impulses contribute to the disease. GABA, a neurotransmitter, fires on GABA receptors on neurons to trigger an inhibitory response. The GABA receptor genes in autism exert their influence via complex gene–gene interactions. Interactions between the GABA receptor genes GABRA4 and GABRB1 have been associated with autism risk.[109] OMIM (Online Mendelian Inheritance in Man) links 263 genes to autism and ASD.

The homeobox transcription factor ENGRAILED 2 (*EN2*) is an ASD susceptibility gene. En2 knockout mice (En2(–/–)) display subtle cerebellar neuropathological changes similar to what has been observed in the ASD brain.[110] The cerebellum is thought to control many of the functions that are impaired in autistic children, such as language and attention span; En2(–/–) mice display behavioral and neurochemical changes, in addition to genetic and neuropathological changes, relevant to ASD. Therefore, these mice may be useful as an animal model of autism. Surveys showed the possibility of prenatal stressors as a potential cause of autism.

Summary

Autism is an urgent public health issue that is increasing at an alarming rate.[111] Abnormal brain development as well as neurodegenerative finding can be traced to pre- and postnatal alterations. Purkinje cell loss is found in autistic individuals compared to controls. A variety of etiology has been proposed for autism including maternal viral infection. Interactions among related genes revealed that complex genetic interactions account for autism risk. The genes involved normally in the dampening of nerve impulses contribute to the disease. Genetic factors play a key role in autism. Based on a maternal prenatal model of autism caused by influenza virus, the early events that trigger this complex cluster of neurological disorders may involve the breach of nasal epithelial cells, which leads to the influx of water which in turn damages the developing neurons during the early stages of brain development. Alternatively neurodegenerative disorders can be caused by the interaction of environmental agents like heavy metals with transport proteins like AQPs and GJP complexes embedded in the neuronal network during synaptogenesis.

Author Contributions

Conceived the concepts: HHPC and RVR. Wrote the first draft of the manuscript: HHPC. Contributed to the writing of the manuscript: RVR and NK. Jointly developed the structure and arguments for the paper: RVR and HHPC. Made critical revisions and approved final version: HHPC. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Prevalence of Autism Spectrum Disorders–Autism and Developmental Disabilities Monitoring Network, United States, 2008. Morbidity and Mortal Weekly Report (MMWR) 2012;61(3). (http://www.cdc.gov/ncbddd/autism/documents/addm-2012-community-report.pdf)
2. Scatena R, Martorana GE, Bottoni P, Botta G, Pastore P, Giardina B. An update on pharmacological approaches to neurodegenerative diseases. *Expert Opin Investig Drugs*. 2007;16:59–72.
3. Cohly HH, Panja A. Immunological findings in autism. *Int Rev Neurobiol*. 2005; 71:317–341.
4. Moy SS, Nadler JJ, Young NB, et al. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. *Behav Brain Res*. 2007;176:4–20.
5. Van Naarden BK, Pettygrove S, Daniels J, et al. Evaluation of a methodology for a collaborative multiple source surveillance network for autism spectrum disorders—autism and developmental disabilities monitoring network, 14 sites, United States, 2002. *MMWR Surveill Summ*. 2007;56:29–40.
6. Minshew NJ. Brief report: brain mechanisms in autism: functional and structural abnormalities. *J Autism Dev Disord*. 1996;26:205–209.
7. Baio J. Prevalence of Autism Spectrum Disorders—Autism and Developmental Disabilities Monitoring Network, 14 Sites, United States, 2008. *Surveillance Summaries*. 2012;61(3):1–19.
8. Lainhart JE, Ozonoff S, Coon H, et al. Autism, regression, and the broader autism phenotype. *Am J Med Genet*. 2002;113:231–237.
9. Plioplys AV, Hemmens SE, Regan CM. Expression of a neural cell adhesion molecule serum fragment is depressed in autism. *J Neuropsychiatry Clin Neurosci*. 1990;2:413–417.
10. Yip J, Soghomonian JJ, Blatt GJ. Decreased GAD67 mRNA levels in cerebellar Purkinje cells in autism: pathophysiological implications. *Acta Neuropathol (Berl)*. 2007;113:559–568.
11. Bethea TC, Sikich L. Early pharmacological treatment of autism: a rationale for developmental treatment. *Biol Psychiatry*. 2007;61:521–537.
12. Hardan AY, Girgis RR, Lacerda AL, et al. Magnetic resonance imaging study of the orbitofrontal cortex in autism. *J Child Neurol*. 2006;21:866–871.
13. Ashwin C, Baron-Cohen S, Wheelwright S, O'Riordan M, Bullmore ET. Differential activation of the amygdala and the 'social brain' during fearful face-processing in Asperger syndrome. *Neuropsychologia*. 2007;45:2–14.
14. Rippon G, Brock J, Brown C, Boucher J. Disordered connectivity in the autistic brain: challenges for the "new psychophysiology". *Int J Psychophysiol*. 2007;63: 164–172.
15. Brown C, Gruber T, Boucher J, Rippon G, Brock J. Gamma abnormalities during perception of illusory figures in autism. *Cortex*. 2005;41:364–376.
16. Toal F, Murphy DG, Murphy KC. Autistic-spectrum disorders: lessons from neuroimaging. *Br J Psychiatry*. 2005;187:395–397.
17. Noriega DB, Savelkoul HF. Immune dysregulation in autism spectrum disorder. *Eur J Pediatr*. 2014;173(1):33–43.
18. Qiu A, Adler M, Crocetti D, Miller MI, Mostofsky SH. Basal ganglia shapes predict social, communication, and motor dysfunctions in boys with autism spectrum disorder. *J Am Acad Child Adolesc Psychiatry*. 2010;49(6):539–551.
19. Konopka G, Friedrich T, Davis-Turak J, et al. Human-specific transcriptional networks in the brain. *Neuron*. 2012;75(4):601–617.
20. Oldham MC, Horvath S, Geschwind DH. Conservation and evolution of gene coexpression networks in human and chimpanzee brains. *Proc Natl Acad Sci U S A*. 2006;103(47):17973–17978.

21. Gokhale SG, Gokhale SS. Genetic and evolutional mechanisms explain 'associated malformations'. *Med Hypotheses*. 2007;69(4):879–883.
22. Buxhoeveden DP, Semendeferi K, Buckwalter J, Schenker N, Switzer R, Courchesne E. Reduced minicolumns in the frontal cortex of patients with autism. *Neuropathol Appl Neurobiol*. 2006;32:483–491.
23. Casanova MF, Buxhoeveden DP, Switala AE, Roy E. Minicolumnar pathology in autism. *Neurology*. 2002;58:428–432.
24. Stanfield AC, McIntosh AM, Spencer MD, Philip R, Gaur S, Lawrie SM. Towards a neuroanatomy of autism: a systematic review and meta-analysis of structural magnetic resonance imaging studies. *Eur Psychiatry*. 2008;23(4):289–299.
25. Courchesne E, Redcay E, Kennedy DP. The autistic brain: birth through adulthood. *Curr Opin Neurol*. 2004;17:489–496.
26. Welsh JP, Yuen G, Placantonakis DG, et al. Why do Purkinje cells die so easily after global brain ischemia? Aldolase C, EAAT4, and the cerebellar contribution to posthypoxic myoclonus. *Adv Neurol*. 2002;89:331–359.
27. Cervos-Navarro J, Diemer NH. Selective vulnerability in brain hypoxia. *Crit Rev Neurobiol*. 1991;6:149–182.
28. Butterworth RF. Pathophysiology of cerebellar dysfunction in the Wernicke-Korsakoff syndrome. *Can J Neurol Sci*. 1993;20(suppl 3):S123–S126.
29. Brorson JR, Manzolillo PA, Gibbons SJ, Miller RJ. AMPA receptor desensitization predicts the selective vulnerability of cerebellar Purkinje cells to excitotoxicity. *J Neurosci*. 1995;15:4515–4524.
30. O'Hearn E, Molliver ME. The olivocerebellar projection mediates ibogaine-induced degeneration of Purkinje cells: a model of indirect, trans-synaptic excitotoxicity. *J Neurosci*. 1997;17:8828–8841.
31. Fonnum F, Lock EA. Cerebellum as a target for toxic substances. *Toxicol Lett*. 2000;11(2–113):9–16.
32. Kang TC, Park SK, Hwang IK, et al. The decreases in calcium binding proteins and neurofilament immunoreactivities in the Purkinje cell of the seizure sensitive gerbils. *Neurochem Int*. 2002;40:115–122.
33. Kish SJ, Young T, Li PP, et al. Elevated stimulatory and reduced inhibitory G protein alpha subunits in cerebellar cortex of patients with dominantly inherited olivopontocerebellar atrophy. *J Neurochem*. 1993;60:1816–1820.
34. Reader TA, Senecal J. Topology of ionotropic glutamate receptors in brains of heterozygous and homozygous weaver mutant mice. *Synapse*. 2001;42:213–233.
35. Pletnikov MV, Rubin SA, Vasudevan K, Moran TH, Carbone KM. Developmental brain injury associated with abnormal play behavior in neonatally Borna disease virus-infected Lewis rats: a model of autism. *Behav Brain Res*. 1999;100:43–50.
36. Ross JF, Switzer RC, Poston MR, Lawhorn GT. Distribution of bismuth in the brain after intraperitoneal dosing of bismuth subnitrate in mice: implications for routes of entry of xenobiotic metals into the brain. *Brain Res*. 1996;725:137–154.
37. Sorensen FW, Larsen JO, Eide R, Schionning JD. Neuron loss in cerebellar cortex of rats exposed to mercury vapor: a stereological study. *Acta Neuropathol (Berl)*. 2000;100:95–100.
38. Warfvinge K. Mercury distribution in the neonatal and adult cerebellum after mercury vapor exposure of pregnant squirrel monkeys. *Environ Res*. 2000;83:93–101.
39. Kenntner N, Tataruch F, Krone O. Heavy metals in soft tissue of white-tailed eagles found dead or moribund in Germany and Austria from 1993 to 2000. *Environ Toxicol Chem*. 2001;20:1831–1837.
40. Sakamoto M, Kakita A, Wakabayashi K, Takahashi H, Nakano A, Akagi H. Evaluation of changes in methylmercury accumulation in the developing rat brain and its effects: a study with consecutive and moderate dose exposure throughout gestation and lactation periods. *Brain Res*. 2002;949:51–59.
41. Stoev SD, Grozeva N, Simeonov R, et al. Experimental cadmium poisoning in sheep. *Exp Toxicol Pathol*. 2003;55:309–314.
42. Piao F, Ma N, Hiraku Y, et al. Oxidative DNA damage in relation to neurotoxicity in the brain of mice exposed to arsenic at environmentally relevant levels. *J Occup Health*. 2005;47:445–449.
43. Riedel CJ, Muraszko KM, Youle RJ. Diphtheria toxin mutant selectively kills cerebellar Purkinje neurons. *Proc Natl Acad Sci U S A*. 1990;87:5051–5055.
44. Saavedra H, De MA, Palestini M. Neuronal changes induced by chronic toluene exposure in the cat. *Arch Ital Biol*. 1996;134:217–225.
45. McDonald JW, Shapiro SM, Silverstein FS, Johnston MV. Role of glutamate receptor-mediated excitotoxicity in bilirubin-induced brain injury in the Gunn rat model. *Exp Neurol*. 1998;150:21–29.
46. Crooks R, Mitchell T, Thom M. Patterns of cerebellar atrophy in patients with chronic epilepsy: a quantitative neuropathological study. *Epilepsy Res*. 2000;41:63–73.
47. Bhatia KP, Brown P, Gregory R, et al. Progressive myoclonic ataxia associated with coeliac disease. The myoclonus is of cortical origin, but the pathology is in the cerebellum. *Brain*. 1995;118(pt 5):1087–1093.
48. Tijssen MA, Thom M, Ellison DW, et al. Cortical myoclonus and cerebellar pathology. *Neurology*. 2000;54:1350–1356.
49. Hadjivassiliou M, Boscolo S, vies-Jones GA, et al. The humoral response in the pathogenesis of gluten ataxia. *Neurology*. 2002;58:1221–1226.
50. Barlow SM, Sullivan FM. Reproductive hazards and industrial chemicals. *Ann Occup Hyg*. 1981;24:359–361.
51. Heaton MB, Mitchell JJ, Paiva M. Amelioration of ethanol-induced neurotoxicity in the neonatal rat central nervous system by antioxidant therapy. *Alcohol Clin Exp Res*. 2000;24:512–518.
52. Yamashita T, Ando Y, Obayashi K, et al. Oxidative injury is present in Purkinje cells in patients with olivopontocerebellar atrophy. *J Neurol Sci*. 2000;175:107–110.
53. Chen P, Peng C, Luff J, et al. Oxidative stress is responsible for deficient survival and dendritogenesis in purkinje neurons from ataxia-telangiectasia mutated mutant mice. *J Neurosci*. 2003;23:11453–11460.
54. Courchesne E, Yeung-Courchesne R, Press GA, Hesselink JR, Jernigan TL. Hypoplasia of cerebellar vermal lobules VI and VII in autism. *N Engl J Med*. 1988;318:1349–1354.
55. Schaefer GB, Thompson JN, Bodensteiner JB, et al. Hypoplasia of the cerebellar vermis in neurogenetic syndromes. *Ann Neurol*. 1996;39:382–385.
56. Benayed R, Gharani N, Rossman I, et al. Support for the homeobox transcription factor gene ENGRAILED 2 as an autism spectrum disorder susceptibility locus. *Am J Hum Genet*. 2005;77:851–868.
57. James SJ, Cutler P, Melnyk S, Jernigan S, Janak L, Gaylor DW, Neubrander JA. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am J Clin Nutr*. 2004;80:1611–1617.
58. James SJ, Melnyk S, Jernigan S, et al. Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am J Med Genet B Neuropsychiatr Genet*. 2006;141B:947–956.
59. Shearer TR, Larson K, Neuschwander J, Gedney B. Minerals in the hair and nutrient intake of autistic children. *J Autism Dev Disord*. 1982;12:25–34.
60. Wecker L, Miller SB, Cochran SR, Dugger DL, Johnson WD. Trace element concentrations in hair from autistic children. *J Ment Defic Res*. 1985;29(pt 1):15–22.
61. Eppright TD, Sanfacon JA, Horwitz EA. Attention deficit hyperactivity disorder, infantile autism, and elevated blood-lead: a possible relationship. *Mo Med*. 1996;93:136–138.
62. Filipek PA, Accardo PJ, Baranek GT, et al. The screening and diagnosis of autistic spectrum disorders. *J Autism Dev Disord*. 1999;29:439–484.
63. Lonsdale D, Shamberger RJ, Audhya T. Treatment of autism spectrum children with thiamine tetrahydrofurfuryl disulfide: a pilot study. *Neuro Endocrinol Lett*. 2002;23:303–308.
64. Holmes AS, Blaxill MF, Haley BE. Reduced levels of mercury in first baby haircuts of autistic children. *Int J Toxicol*. 2003;22:277–285.
65. Fido A, Al-Saad S. Toxic trace elements in the hair of children with autism. *Autism*. 2005;9:290–298.
66. Dean SL, McCarthy MM. Steroids, sex and the cerebellar cortex: implications for human disease. *Cerebellum*. 2008;7(1):38–47.
67. Fatemi SH. Reelin glycoprotein in autism and schizophrenia. *Int Rev Neurobiol*. 2005;71:179–187.
68. Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect*. 2000;108:511–533.
69. Kidd PM. Autism, an extreme challenge to integrative medicine. Part: 1: the knowledge base. *Altern Med Rev*. 2002;7:292–316.
70. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*. 2005;57:67–81.
71. Beversdorf DQ, Manning SE, Hillier A, et al. Timing of prenatal stressors and autism. *J Autism Dev Disord*. 2005;35:471–478.
72. Ingram JL, Peckham SM, Tisdale B, Rodier PM. Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. *Neurotoxicol Teratol*. 2000;22:319–324.
73. Fatemi SH, Pearce DA, Brooks AI, Sidwell RW. Prenatal viral infection in mouse causes differential expression of genes in brains of mouse progeny: a potential animal model for schizophrenia and autism. *Synapse*. 2005;57:91–99.
74. Campbell JD, Elford RW, Brant RF. Case-control study of prenatal ultrasonography exposure in children with delayed speech. *CMAJ*. 1993;149:1435–1440.
75. Edwards MJ. Apoptosis, the heat shock response, hyperthermia, birth defects, disease and cancer. Where are the common links? *Cell Stress Chaperones*. 1998;3:213–220.
76. Zoghbi HY. Postnatal neurodevelopmental disorders: meeting at the synapse? *Science*. 2003;302:826–830.
77. Schumann CM, Amaral DG. Stereological analysis of amygdala neuron number in autism. *J Neurosci*. 2006;26:7674–7679.
78. Yao L, Korteweg C, Hsueh W, Gu J. Avian influenza receptor expression in H5N1-infected and noninfected human tissues. *FASEB J*. 2008;22(3):733–740.
79. Shi L, Fatemi SH, Sidwell RW, Patterson PH. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J Neurosci*. 2003;23:297–302.
80. Bolte ER. Autism and *Clostridium tetani*. *Med Hypotheses*. 1998;51:133–144.

81. Fatemi SH, Reutiman TJ, Folsom TD, Sidwell RW. The role of cerebellar genes in pathology of autism and schizophrenia. *Cerebellum*. 2008;7(3):279–294.
82. Senne DA. Avian influenza in North and South America, 2002–2005. *Avian Dis*. 2007;51:167–173.
83. Alexander DJ. Summary of avian influenza activity in Europe, Asia, Africa, and Australasia, 2002–2006. *Avian Dis*. 2007;51:161–166.
84. Gu J, Xie Z, Gao Z, et al. H5N1 infection of the respiratory tract and beyond: a molecular pathology study. *Lancet*. 2007;370:1137–1145.
85. Matrosovich MN, Matrosovich TY, Gray T, Roberts NA, Klenk HD. Human and avian influenza viruses target different cell types in cultures of human airway epithelium. *Proc Natl Acad Sci U S A*. 2004;101:4620–4624.
86. Thompson CI, Barclay WS, Zambon MC, Pickles RJ. Infection of human airway epithelium by human and avian strains of influenza a virus. *J Virol*. 2006;80: 8060–8068.
87. Horvath K, Perman JA. Autism and gastrointestinal symptoms. *Curr Gastroenterol Rep*. 2002;4:251–258.
88. Mostafa GA, Al-Ayadhi LY. The relationship between the increased frequency of serum antineuronal antibodies and the severity of autism in children. *Eur J Paediatr Neurol*. 2012;16(5):464–468.
89. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood–brain barrier. *Nat Rev Neurosci*. 2006;7:41–53.
90. Ichiyama T, Morishima T, Kajimoto M, Matsushige T, Matsubara T, Furukawa S. Matrix metalloproteinase-9 and tissue inhibitors of metalloproteinases 1 in influenza-associated encephalopathy. *Pediatr Infect Dis J*. 2007;26:542–544.
91. Kido H, Yao D, Le TQ, Tsukane M, Chida J. Analysis of SNPs and enzymatic disorder in the patients of influenza-associated encephalopathy: disorder of fatty acid metabolism in mitochondria induced by high fever. *Nippon Rinsho*. 2006;64: 1879–1886.
92. Yao D, Chen Y, Kuwajima M, Shiota M, Kido H. Accumulation of mini-plasmin in the cerebral capillaries causes vascular invasion of the murine brain by a pneumotropic influenza A virus: implications for influenza-associated encephalopathy. *Biol Chem*. 2004;385:487–492.
93. Hallene KL, Oby E, Lee BJ, et al. Prenatal exposure to thalidomide, altered vasculogenesis, and CNS malformations. *Neuroscience*. 2006;142:267–283.
94. Willis CL, Leach L, Clarke GJ, Nolan CC, Ray DE. Reversible disruption of tight junction complexes in the rat blood-brain barrier, following transitory focal astrocyte loss. *Glia*. 2004;48:1–13.
95. Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta*. 2008;1778(3): 660–669.
96. Neijssen J, Pang B, Neefjes J. Gap junction-mediated intercellular communication in the immune system. *Prog Biophys Mol Biol*. 2007;94:207–218.
97. Elias LA, Wang DD, Kriegstein AR. Gap junction adhesion is necessary for radial migration in the neocortex. *Nature*. 2007;448:901–907.
98. Sohl G, Maxeiner S, Willecke K. Expression and functions of neuronal gap junctions. *Nat Rev Neurosci*. 2005;6:191–200.
99. Torrente F, Anthony A, Heuschkel RB, Thomson MA, Ashwood P, Murch SH. Focal-enhanced gastritis in regressive autism with features distinct from Crohn's and Helicobacter pylori gastritis. *Am J Gastroenterol*. 2004;99:598–605.
100. Inagaki-Ohara K, Sawaguchi A, Suganuma T, Matsuzaki G, Nawa Y. Intraepithelial lymphocytes express junctional molecules in murine small intestine. *Biochem Biophys Res Commun*. 2005;331:977–983.
101. Tabuchi K, Blundell J, Etherton MR, et al. A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. *Science*. 2007;318: 71–76.
102. Hendry J, DeVito T, Gelman N, et al. White matter abnormalities in autism detected through transverse relaxation time imaging. *Neuroimage*. 2006;29: 1049–1057.
103. Lehmann GL, Gradilone SA, Marinelli RA. Aquaporin water channels in central nervous system. *Curr Neurovasc Res*. 2004;1:293–303.
104. Sobue K, Asai K, Katsuya H. Aquaporin water channels in the brain and molecular mechanisms of brain edema. *Nippon Rinsho*. 2006;64:1181–1189.
105. Saadoun S, Papadopoulos M, Bell B, Krishna S, Davies D. The aquaporin-4 water channel and brain tumour oedema. *J Anat*. 2002;200:528.
106. Gorelick DA, Praetorius J, Tsunenari T, Nielsen S, Agre P. Aquaporin-11: a channel protein lacking apparent transport function expressed in brain. *BMC Biochem*. 2006;7:14.
107. Rajnarayanan R, Varadharajan S, Isokpehi R, Cohly H. Potential role of cation-aquaporin interactions in autism. In: Biomedical Science & Engineering Conference, 2009. BSEC 2009. First Annual ORNL. 1–4, March 18–19, 2009; doi: 10.1109/BSEC.2009.5090494
108. Cohly HP, Isokpehi RD, Agrawal SA, Rajnarayanan RV. Gene expression dynamics of aquaporins and gap junction proteins in human malaria infection. Proceedings of the International Conference on Environmental Parasitology and Community Health Care Initiatives, Agra, India (ENPARACOHI-2007). 2007:20–23.
109. Ma DQ, Whitehead PL, Menold MM, et al. Identification of significant association and gene–gene interaction of GABA receptor subunit genes in autism. *Am J Hum Genet*. 2005;77:377–388.
110. Cheh MA, Millonig JH, Roselli LM, et al. En2 knockout mice display neurobehavioral and neurochemical alterations relevant to autism spectrum disorder. *Brain Res*. 2006;1116:166–176.
111. CDC. *How Common are Autism Spectrum Disorders (ASD)*. Centers for Disease Control and Prevention; 2007. Ref Type: Electronic Citation.