EXPERT REVIEWS

Autoimmune and gastrointestinal dysfunctions: does a subset of children with autism reveal a broader connection?

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A large number of autoimmune disorders have a gastrointestinal (GI) dysfunction component that may interplay with genetic, hormonal, environmental and/or stress factors. This narrarive review investigates possible links between autism, immune system abnormalities and GI symptoms in a subgroup of children with autism. A literature search on Medline (1950 to September 2010) was conducted to identify relevant articles by using the keywords 'autism and gastrointestinal' (71 publications) and 'autism and immune' (237 publications), cross-referencing and general searching to evaluate the available literature on the immunological and GI aspects of autism. Sufficient evidence exists to support that a subgroup of children with autism may suffer from concomitant immune-related GI symptoms.

Keywords: abdominal distension • autism • autoimmune • constipation • diarrhea • food allergy • food sensitivity • gastrointestinal • genetic • permeable gut

Autism

Autism is a neurodevelopmental disorder that usually emerges in children prior to 3 years of age. Often lasting a lifetime, the condition starts with a loss of developmental milestones in three domains – social, communication and movement. The diagnostic indicators of autism are core behavioral symptoms that may include language delay, poor verbal communication, avoidance of physical or eye contact, a decreased ability to bond emotionally, lack of socialization leading to isolated play, shrieking or laughing for no apparent reason and repetitive behaviors such as spinning or head banging [1]. Seizures and mental retardation may also occur in a subset of children with autistic disorder to varying degrees.

Diagnosis

Since autism was not originally linked to an underlying pathophysiology, diagnosis is defined under the Diagnostic Manuals of the American Psychiatry Association (DSM-IV-TR) [2,3]. Autistic disorder is listed under the broader umbrella of autism spectrum disorders (ASD) that includes:

- Autistic disorder;
- Asperger's disorder;
- Childhood disintegrative disorder;
- Rett's disorder;
- Pervasive developmental disorder – not otherwise specified.

Diagnosis is based on a cluster of symptoms classifying autism as a syndrome in the field of psychiatry. As a result, only developmental behavioral milestones and behavioral symptoms are evaluated to reach the diagnosis. There are no specific laboratory markers, imaging findings or electroencephalography results to serve as biological markers for ASD [4].

Prevalence

The prevalence of autism in industrialized nations has been increasing faster than genetic causes or changes in diagnosis definitions can explain. The prevalence rate is one in 100, but it can range from one in 78 to one in 500 children, depending upon country and region. Two decades ago, the prevalence was estimated as

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one in 1000 [5]. Boys are four times more likely to be diagnosed than girls [6].

Genetic factors

While the etiology of ASD still remains unknown, the higher rate of autism in boys suggests a genetic component [7,8]. Siblings are also affected at higher rates than chance (4.5%) and especially identical (monozygotic) twins (60–90%), but not so much dizygotic twins [9]. The genetic link is further supported by other genetic conditions whose symptoms resemble ASD, including fragile X syndrome, tuberous sclerosis, 15q duplications, Rett's syndrome and untreated phenylketonuria [10]. These observations are confirmed by researchers revealing multiple copy number variations in individuals with autism spectrum disorder [11].

Environmental factors

Physiologically, underconnectivity from thinning of the corpus callosum and impaired myelination of white matter have been suggested as significant etiologies for autism [12]. Despite strong evidence for a genetic factor in autism, Pardo and Eberhardt stated, "it is clear that genetics alone do not determine the entire ASD phenotype, and that other nongenetic factors must play roles as modifiers of processes determined by genetic susceptibility" [13]. Since there cannot be a 'genetic epidemic', the increasing rates of autism in developed countries suggest environmental factors that have not yet been elucidated [14].

Purpose of this paper

This narrarive review investigates the possible existence and interconnections of two factors, specifically immune system abnormalities and gastrointestinal (GI) symptoms in a subgroup of children with autism [15].

Methods

A literature search on Medline (including articles that were published from 1950 up to September 2010) was conducted to identify relevant articles by using the keywords 'autism and gastrointestinal' and 'autism and immune', as well as cross-referencing and general searching in order to evaluate the available literature on the immunological and GI aspects of autistic disorder. A narrative review was then written based on a summary compilation of these articles.

Results

Approximately 171 publications existed strictly under the combination keywords of 'autism and gastrointestinal', and 237 publications were found using the keywords of 'autism and immune'. Additional articles were obtained during the search, creating a literature review focusing on a possible link between autism, autoimmunity and GI symptoms. Autism will now be reviewed in terms of a relationship, if any, to the immune and GI systems.

Autism & the immune system

The relationship between autism and immunity surfaced approximately 40 years ago when higher rates of autoimmune conditions were observed in family members. Comi *et al.* reported that 46% (n = 28/61) of autism patient families had two or more members with autoimmune disorders such as Type I diabetes, rheumatoid arthritis, hypothyroidism and systemic lupus erythematosus compared with only 26% (n = 12/46) of controls [16]. Researchers of a more recent study looked at all the children born in Denmark from 1993 to 2004 (689,196 children) and found a total of 3325 children diagnosed with ASDs, of which 1089 had an infantile autism diagnosis [17]. They reported an increased risk of ASDs for children with a maternal history of rheumatoid arthritis and celiac disease. An increased risk of infantile autism occurred with a family history of Type 1 diabetes.

Reviews of immune abnormalities in children with autism have been previously published [13,18–23]. These papers support that some autism polymorphisms may relate to a congenital immune deficiency and/or faulty autoimmune mechanisms. This is based on reports of altered autoantibody production, other immune imbalances and not unexpectedly, higher rates of infections and food allergies in children with ASD. A representative selection of these studies and possible mechanisms of an altered immune–GI link are now discussed, representing the tip of the iceberg in the growing field of research surveying abnormal immune responses in children with ASD.

Altered autoantibody production

The interest in immunity among individuals diagnosed with autism began in the early 1970s. At the same Johns Hopkins hospital where 'early infantile autism' was coined by Leo Kanner almost half a century ago, Money *et al.* stated, that in addition to genetics, autism "might conceivably be attributed also to a primary effect of autoimmune impairment from the formation of autoantibodies against the CNS" [24].

One of the first studies showing altered autoantibody production in autism was by Stubbs who found undetectable titers in five of 13 autistic children against a rubella vaccine [25]. This was later supported by Libbey *et al.*, reporting "a significant number (15/59) of autism subjects (classic and regressive onset combined) who had a very low or no antibody titer against rubella virus, compared with a combined control/Tourette's group (2/49)" [26]. The researchers suggested that an altered immune response existed in these children; however, subject size is inherently small when studying children with autism who also vary in age and type of diagnosis. Some researchers reported significantly higher IgG autoantibodies against neuronal axon filament protein and glial fibrillary acidic protein among children diagnosed with autism compared with children diagnosed with mental retardation [27,28]. It was suggested that "clinically, these autoantibodies may be related to autoimmune pathology in autism." Rout and Dhossche reported that "selective loss of Purkinje cells and cerebellar atrophies are the neurological abnormalities most consistently found in persons diagnosed with autism" [29]. Purkinje cells are involved in motor coordination, working memory and learning. The authors postulated that the severity of ASD was based on the extent of Purkinje cell loss, triggered by glutamate acid decarboxylase antibody. Both Jepson *et al.* and Wills *et al.* thoroughly reviewed the role of autoantibodies in ASD [30,31].

Other immune imbalances

Other immune abnormalities include, but are not limited to, cell-mediated response to myelin, increased proinflammatory cytokines [32], decreased T cells [33], low natural killer (NK) cell activity [33,34] and altered levels of immunoglobulins [35]. These will now each be briefly discussed.

Cell-mediated response to myelin

The brains of children with autism may be targeted by the cell-mediated response. Weizman *et al.* conducted a test where "cell-mediated immune response to human myelin basic protein was studied by the macrophage migration inhibition factor test" [27]. The researchers of this older study were not clear if it was the myelin being targeted by antigen-specific immune responses. However, it was stated that the "results indicated the existence of a cell-mediated immune response to brain tissue in the syndrome of autism."

Innate immunity activated inflammation

Vargas *et al.* studied brain tissues obtained from autistic children at autopsy and independently reported inflammation in the cerebral cortex and white matter characterized by astroglial and neuroglial activation [36]. They concluded that their findings "indicate that innate neuroimmune reactions play a pathogenic role in an undefined proportion of autistic patients" [36].

Increased proinflammatory cytokines

Li *et al.* detected significantly increased proinflammatory cytokines (TNF-α, IL-6 and granulocyte–macrophage colony-stimulating factor [GM-CSF]), Th1 cytokine (IFN-γ) and chemokine (IL-8) in the brains of eight ASD patients compared with eight controls [32]. Although their sample size was small, they concluded that localized brain inflammation and autoimmune disorder may be involved in the pathogenesis of ASD.

Decreased number of T cells

Warren *et al.* reported that "a study of 31 autistic patients revealed reduced responsiveness in the lymphocyte blastogenesis assay to PHA, concanavalin A and pokeweed mitogen; decreased numbers of T lymphocytes; and an altered ratio of helper to suppressor T cells" [33]. Again, as in most studies with autistic patients, the sample size was small, but they suggested that one of the genes (*C4B*, involved in removing pathogens such as viruses and bacteria) from the body was more frequently found in it's deficient form (*C4B* null allele – no C4B protein produced) in children with ASD.

Low NK cell activity

Very few studies have explored NK activity in autism. Vojdani *et al.* measured NK activity in the 1027 blood samples from autistic children obtained from ten clinics and compared them to 113 healthy controls [37]. After a correction factor, 45% of the subgroup of children with autism exhibited low NK cell activity, possibly related to low intracellular levels of glutathione, IL-2 and IL-15.

Serum immunoglobulins

It is established that IgA levels change with age, a factor that is often not integrated in studies with autistic children. With this in mind, one research group reported autistic children having higher levels of IgG and IgM, while having lower levels of IgA [35]. Conversely, Heuer *et al.* reported the opposite – that there were lower levels of plasma IgG and IgM in autistic individuals compared with controls (no significant IgA differences), but they also correlated the immunoglobulin levels with behavioral severity [38]. If ASD children have lower serum levels of IgA, it would be of significance because IgA is a major immunoglobulin in the GI tract with a pivotal role in oral tolerance [39]. Santaella *et al.* reported a high incidence of allergies (71%) in IgA-deficient patients and all of whom had food sensitivities [39]. Although Santaella *et al.* had age- and gender-matched controls, many of studies examining IgA or other immunoglobulin levels in autistic children do not.

Higher rates of infection

Given the above immune abnormalities, it is not surprising that higher rates of infection are found in ASD children. In 1943, Kanner, who originated the 'early infantile autism' diagnosis, documented symptoms of higher rates of infections: "large tonsils and adenoids … in bed often because of colds, bronchitis, streptococcus infection … repeated colds and otitis media" [40]. Since then, researchers have established that autistic children have higher rates of otitis media [41,42] involving repeated rounds of antibiotics [42], allergies [39], delayed-type food allergies [43], pediatrician visits [44,45] and hospital visits [44,45].

Jyonouchi *et al.* noted that "among patients with ASD evaluated in our clinic (Pediatric Allergy/Immunology Clinic), there appears to be a subset that can be clinically distinguished from other ASD children because of frequent infections (usually viral) accompanied by worsening behavioral symptoms and/or loss/decrease of acquired skills." They also mentioned parents of ASD children reporting frequent viral infections, pharyngitis, recurrent otitis media and chronic sinusitis [43].

Higher rates of food allergies

Gurney *et al.* found not only higher rates of respiratory and skin allergies being reported by parents of children with autism (not specified as whether IgE-mediated or not), but also food allergies [45]. When the ASD viral-prone children in the Jyonouhci *et al.* study were compared with non-ASD controls with asthma (instead of the 23 nonviral-prone ASD children), they were found to have more food allergies. In fact, the percentage of non-IgE mediated food allergies in the viral ASD group (89%) and nonviral ASD group (78%) were much higher than in non-ASD controls with asthma (0%) [43]. This study actually supported higher rates of delayed-type food allergies in ASD children (89 and 78%) that were similar to the group with food allergies (92%). Primary immunodeficiency was 19% in the viral ASD group compared with 0% in the asthma controls.

Possible mechanisms of altered immune–GI link

Genetics appears to play a role in what appears to be an altered immune function in children with autism, followed by a number

of hypothesized mechanisms that range from not plausible to possible. These include, but are not limited to, inadequate immune response to pathogen infections, possible prenatal exposure to environmental factors and intestinal flora imbalance.

Inadequate immune response to pathogen infections

Do pathogens play a role in the immune dysfunction of children with ASD? Although debated extensively, Singh and Jensen suggested that "virus-induced autoimmunity may play a causal role in autism" [28]. They reported significantly higher levels of measles antibody (but not mumps or rubella antibodies) in autistic children compared with normal children, or siblings of autistic children. Although the western blot results of this paper were questionable, they suggested that autistic children have a hyperimmune response to measles virus that may be a sign of an abnormal immune reaction to the vaccine strain.

The debate continues as to whether the immune abnormality in ASD children is hyperactive or hypoactive. For example, Kawashti *et al.* studied 30 autistic children (aged 3–6 years) and 30 nonautistic psychologically symptom-free siblings and were surprised to find that "circulating anti-measles, anti-mumps and anti-rubella IgG were positive in only 50, 73.3 and 53.3%, respectively, as compared with 100% positivity in the control group" [46]. The authors concluded that "…a deficient immune response to measles, mumps and rubella vaccine antigens might be related to autism."

The above research results are very limited in number and cannot be used to make any conclusions. However, they do raise the question of which comes first – the immune dysfunction possibly related to genetics or the viral infection, and/or does a virus or any infectious agent cause a cascading series of problems in a genetically immunocompromised individual? Van Gent *et al.* suggested two "related conceptual frameworks: a viral and an autoimmune hypothesis" [47].

Prenatal exposure

Another viral theory may involve prenatal exposure. Maternal viral infection is known to increase the risk for schizophrenia and autism in the offspring of animal models [48]. Both Shi *et al.* and Fatemi *et al.* reported that infecting pregnant mice with influenza virus resulted in brain changes similar to those observed in autism and that myelination genes may be involved [49,50]. Fatemi *et al.* also reported that "administration of human influenza virus in pregnant mice led to significant gene alterations in the offsprings' frontal, hippocampal and cerebellar cortices. Brain imaging revealed significant atrophy in several brain areas and white matter thinning in corpus callosum" [51].

In terms of human studies, second trimester exposure to respiratory infection is associated with a significantly increased risk of schizophrenia [52]. Very few studies exist that have investigated the association between human maternal infection during pregnancy and the development of ASDs in the offspring. Atladóttir *et al.* studied nationwide registers of children born in Denmark between 1980 and 2005 who were diagnosed with ASD and general maternal infection during pregnancy [53]. Although no association was found during the total period of pregnancy, admission to the hospital due to maternal viral infection in the first trimester and maternal bacterial infection in the second trimester were found to be associated with diagnosis of ASDs in the offspring. They suggested that early prenatal viral infection increases the risk of ASDs, but that more research focusing solely on influenza is needed.

Some researchers have documented abnormal prenatal immune response in mothers against the fetus [54]. Dalton *et al.* tested the serum of a mother of an ASD child and found serum antibodies binding to rodent Purkinje cells and other neurons [55]. When this mother's serum was injected into pregnant mice, their offspring exhibited negative behavior (motor coordination problems, altered exploration) and structural changes in the cerebellum. The serum injected from mothers of non-ASD children resulted in no changes in their offspring [55]. Maternal antibodies were found against the autistic children's lymphocytes in six out of 11 mothers [56]. In another study, Zimmerman *et al.* found that the mothers of ASD children, but not those from control mothers, produced antibodies against prenatal, but not postnatal or adult rat brain tissue [57]. The researchers concluded that autoantibodies may cross the placenta and alter fetal brain development. Braunscheweig *et al.* also suggested an association between the transfer of IgG autoantibodies during pregnancy and the risk of developing autism [58]. They observed reactivity against fetal, but not adult, brain tissue in 11% (n = 7/61) of mothers of children with autism.

Intestinal flora imbalance

An untested hypothesis is whether or not intestinal flora imbalance, possibly triggered by repeated antibiotic use for recurrent ear infections or other reasons, is a contributing factor in GI problems. Does an abnormal immune system due to faulty genes fail to adequately eliminate the pathogen (bacterial, viral, yeast and/or parasite) from their system? Or perhaps a deficient, but activated immune system continues unabated, resulting in a continuous immune response producing inflammation? The enzymes along the brush border of the small intestine may then be compromised, and the affected children may then have trouble completely digesting certain foods because of a compromised enzyme concentration in the inflamed intestinal wall. Reduced disaccharidase (lactase, sucrase and trehalase) activity has been reported to occur on the jejunal mucosa of patients with ulcerative colitis and Crohn's disease [59].

Autism & the GI system

The importance of summarizing research on immune dysfunction in children with autism is that it provides a plausible explanation for occasionally reported GI dysfunction. The majority of autoimmune disorders are related to GI symptoms. Could this be a link not only in autism, the tip of the iceberg, but in other autoimmune conditions that, similar to autism, to this day have their origin's shrouded in mystery? The topic remains controversial, so we now explore research refuting and supporting an autism–GI link, possible mechanisms, GI dysfunctions in other developmental delay disorders and other autoimmune conditions related to GI dysfunction.

Researchers refuting an autism–GI link

Ibrahim *et al.* sought to determine whether autistic children had any more visits to the hospital for GI symptoms than nonautistic children [60]. They conducted a retrospective study of medical records (from 1976 to 1997) identifying 121 children with autism matched in a 1:2 ratio with a patient of the Mayo Clinic with the same age, gender and date of registration. They found a significantly greater prevalence of constipation and 'feeding issues and food selectivity' among children with autism. However, they concluded that "data suggest that a neurobehaviorial rather than a primary organic GI etiology may account for the higher incidence of these GI symptoms in children with autism" [60]. Their 'conclusion' was then promoted nationally through a press release reaching Cable News Network (CNN) and other national media outlets that this study finally puts to rest any link between GI symptoms and autism and that parents do not need to place their children on any restrictive diets. Such a conclusion potentially jeopardizes a subset of children who may have GI symptoms that need to be treated. Serious limitations of Ibrahim's study were addressed in our previous publication [61].

Epidemiological studies, not actual testing of patients with autism, failed to find an association of autism with GI symptoms [62,63]. A third study failed to link celiac disease with autism [64]. Nevertheless, despite four contrary studies, some research supports that a subset of children with autism experience GI problems, which will now be explored.

Research supporting an autism–GI link

A relationship between autism and GI symptoms was first reported by Goodwin *et al.* in 1971, in which anecdotal reports by parents suggested that autistic children suffered GI tract problems related to food sensitivities [15]. Internal GI symptoms included the classic food sensitivity symptoms of chronic diarrhea, abdominal discomfort and distension, excessive gas [65] and constipation [66].

It is well accepted that ASD children often have oral motor delays and are selective eaters, which can lead to food refusal and poor food intake [67–70]. The link between autism and GI in terms of prevalence of GI problems, reported GI examinations, possible mechanisms and GI problems in other developmental delay disorders is now addressed. Other autoimmune conditions related to GI dysfunction are then briefly discussed.

Prevalence of GI problems

Researchers vary widely in their reported percentage of autistic children affected by GI problems but a recent review in *Pediatrics* reported that the prevalence of GI symptoms in children with ASD range widely from 9 to 91%. This was based on 11 studies that together give an approximate prevalence of 40% [71]. GI symptoms were defined differently by the researchers of each study but could include constipation, diarrhea, bloating, belching, abdominal pain, reflux, vomiting and/or flatulence. It is interesting to note that D'Eufemia *et al.* observed that 43% (9/21) of children with ASD had altered intestinal permeability (leaky gut) compared with zero of controls (0/40) [72].

In 2001, an independent evaluation by Lightdale *et al.* reported that almost 50% of the 500 parents surveyed conveyed that their children had loose stools or frequent diarrhea [73]. Levy *et al.* also reported that 44% (26/59) of the ASD children completing stool diaries in their study had abnormal stool consistencies (bulky or loose/mushy) [74]. Valcenti-McDermott *et al.* supported this research when they reported that their survey of parents of 100 children with ASD (gastrointestinal questionnaire and a familial autoimmune questionnaire) resulted in "children with language regression more frequently exhibiting an abnormal stool pattern (40 vs 12%) and increased family history of celiac disease or inflammatory bowel disease (24 vs 0%)" [75]. This again suggests a possible link between autoimmune/inflammatory GI conditions and autism.

Reported GI examinations

Although physical examinations of the GI tract are invasive and not frequently performed in children with ASD, GI abnormalities have been reported. Based on these findings, Lightdale and colleagues suggested that "lymphoid nodular hyperplasia of the terminal ileum, mild colitis, mild duodenitis and altered intestinal permeability have been purported to be more prevalent in autistic than nonautistic populations" [73]. A review article by White stated that "the results … taken together suggest that significant and widespread GI pathophysiology may accompany autism, at least within a subpopulation of patients" [4]. Specific studies are detailed in White's review of intestinal pathophysiology in autism, and now these and a few selected others are briefly highlighted in chronological order.

Despite the controversial measles–autism link [76] by Wakefield *et al.* that led to a prominent, public retraction of their *Lancet* article [77], their group was the first to conduct ileocolonoscopies of children with 'regressive developmental disorder' with a history of intestinal symptoms (diarrhea, abdominal pain, bloating and food intolerance). All 12 children had intestinal abnormalities, ranging from lymphoid nodular hyperplasia to aphthoid ulceration. They were the first to document their observations with photographs and coin the term 'ileal-lymphoid-nodular hyperplasia, nonspecific type of colitis', known elsewhere as 'autistic colitis'. Despite photographs, this observation continues to be debated; however, other researchers have yet to conduct a similar study to support or refute their results. Histology showed patchy chronic inflammation in the colon in 11 children and reactive ileal lymphoid hyperplasia in seven, but no granulomas.

The following year, Horvath conducted upper GI endoscopies of 36 children with ASD and reported that histologic examination revealed grade I or II reflux esophagitis in 69.4% (25/36), chronic duodenitis in 67% (24/36) and chronic gastritis in 42% (15/36) [65]. The number of Paneth's cells in the duodenal crypts was significantly elevated in autistic children compared with nonautistic control subjects. It could be speculated that some digestive symptoms are related to the low intestinal carbohydrate digestive enzyme activity reported in 58.3% of the children (21/36), although there was no abnormality found in pancreatic function.

Wakefield *et al.* continued their studies by conducting an ileocolonoscopy and biopsy on 60 affected children (3–16 years of age; 50 ASD patients that included 12 children from earlier 1998 study, five Asperger's syndrome, two disintegrative disorder, one attention-deficit/hyperactivity disorder, one schizophrenia and one dyslexia) [78]. White indicated that all but one of the children with this wide array of disorders had GI symptoms, including abdominal pain, constipation, diarrhea and bloating [4]. Ileal lymphonodular hyperplasia was present in 93% (54/58) of affected children and in 14.3% (5/35) controls. Colonic lymphonodular hyperplasia was present in 30% (18/60) of affected children versus 5.4% (2/37) controls. Chronic colitis was identified in 88% (53/60) of affected children compared with 4.5% (1/22) of controls and in 20 out of 20 (100%) with ulcerative colitis (UC). Histologically, reactive follicular hyperplasia was present in 88.5% (46/52) of ileal biopsies from affected children, compared with 29% (4/14) with UC, and none in noninflammatory bowel disease controls. Active ileitis was present in 8% (4/51) of affected children but not in controls. Scores of frequency and severity of inflammation were significantly greater in both affected children and those with UC, compared with controls ($p < 0.001$).

Torrente *et al.* studied gastric biopsies in 25 ASD children and compared them to ten subjects with Crohn's disease, ten with *Helicobacter pylori* infection and ten normal controls [79]. They stated that "Horvath *et al.*'s initial report of gastritis in autistic children, and … demonstrated a novel form of focal gastritis dominated by $CD8^+$ T cells … with a pattern of lymphocyte infiltration more similar to that recently identified in Crohn's disease."

Ashwood *et al.* obtained duodenal and colonic biopsies from 21 ASD children and 65 developmentally normal pediatric controls, of which 38 had signs of histological inflammation [80]. They reported a consistent profile in ASD children of $CD3^+$ lymphocyte cytokines in the small and large intestinal mucosa involving increased pro-inflammatory and decreased regulatory activities. Specifically, they found elevated duodenal and colonic mucosal $CD3^+$ lymphocyte counts ($p < 0.03$). In the duodenum, the proportion of lamina propria (LP) and epithelial $CD3^+TNF\text{-}\alpha^+$ cells in ASD children was significantly greater than in noninflamed controls ($p < 0.002$). LP, epithelial $CD3^+IL\text{-}2^+$, $CD3^+IFN\text{-}\gamma^+$ and epithelial $CD3^+IL\text{-}4^+$ cells were more numerous in ASD children than in noninflamed controls ($p < 0.04$). By contrast, $CD3^+IL\text{-}10^+$ cells were fewer in ASD children than in noninflamed controls ($p < 0.05$). In the colon, LP $CD3^+TNF\text{-}\alpha^+$ and $CD3^+IFN\text{-}\gamma^+$ cells were more frequent in ASD children than in noninflamed controls ($p < 0.01$).

It could be suggested that the above observations of GI problems in a subset of children with autism suggest that GI symptoms may occur at some point in the course of their condition or at least in a subset of children. If so, what are the possible mechanisms hypothesized behind these changes?

Possible mechanisms

Numerous theories have surfaced as to the cause of GI symptoms in a subset of children with autism. Other than genetics serving as an important factor in autism, a few other influences (not causes) that are suggested in the literature include increased intestinal permeability, abnormal antibodies and the opioid theory.

Increased intestinal permeability

The aforementioned abnormal GI problems physically observed in a subset of children with ASD suggest an increased risk for intestinal lesions and inflammation. The intestine's natural protection is its protective mucosal barrier and tight junctions between epithelial cells that discourage the absorption of anything into the bloodstream except the smallest components of digestion such as monosaccharides, amino acids, glycerol, fatty acids, minerals, vitamins and other smaller non-nutrients of dietary origin. However, it is hypothesized that intestinal inflammation, regardless of the source (e.g., Crohn's disease, UC, irritable bowel syndrome, celiac disease or infection from microorganisms), compromises the tight junctions, thus allowing larger than normal substances to cross through the intestinal wall and into the blood stream. The portion of the immune system located within the intestines then reacts to the 'foreign' substances in a classic inflammatory response. Researchers have been investigating the possible role of intestinal permeability in the development of celiac disease and other autoimmune disorders, as explained by Fasano [81]. Turner also provides an excellent review on intestinal mucosal barrier function [82]. Intestinal inflammation may very well compromise the enzymes along the brush border of the intestinal wall that further interfere with digestion, and a continuing cycle of immune-modulated inflammation.

To test this theory and determine whether 21 children with ASD had increased intestinal permeability, D'Eufemia *et al.* compared them to 40 healthy age-matched controls [72]. They administered a standard sugar permeability test consisting of the oral administration of mannitol, an easily absorbed monosaccharide, and lactulose, a disaccharide that should not be absorbed owing to its size. After entering the blood stream, these sugars are excreted in the urine where the mannitol:lactulose ratio is calculated, thus determining the gut's degree of 'leakiness' [83]. Nine out of 21 children (43%; 4–16 years of age) with ASD had increased intestinal permeability.

In the same way that other researchers do not suggest that GI disease is the cause of autism, we are not suggesting that intestinal permeability is the cause of GI problems in certain autoimmune conditions. Rather, intestinal permeability is one step in a long continuum of steps leading to GI dysfunction and other related symptoms.

Abnormal antibody levels

Perhaps an inflamed gut, if present in autistic children, results in antibodies being formed against certain food components as a form of natural protection? An inflamed condition associated with increased absorption of macromolecules can lead to increased production of IgG antibody against common dietary proteins. Kawashiti *et al.* studied 30 autistic children (aged 3–6 years) and 30 nonautistic psychologically-symptom free siblings as controls [46]. They detected high seropositivity for autoantibodies to dietary proteins, specifically, casein (83% autistic vs 10%

control) and gluten (50% autistic vs 7% control). Reichelt *et al.* also detected higher levels of IgA against dietary gluten [84], and Lucarelli *et al.* found similarly high levels against the milk proteins of casein, lactalbumin and β-lactoglobulin [85]. When placed on a gluten-free/casein-free diet, researchers "noticed a marked improvement in the behavioral symptoms of patients after a period of 8 weeks on an elimination diet." Their results led them to suggest "a relationship between food allergy and infantile autism" even though IgG antibody is not considered to be closely associated with the pathogenesis of either IgE- or non-IgE-mediated food allergy.

Opioid theory

The theory stemming from schizophrenia research crossing over to autism is known as the opioid theory. Although not fully supported by the scientific community, it was suggested that the partial digestion of gluten (gliadin) and casein results in gliadomorphins and casomorphins, respectively. Apparently, proteolytic enzymes in the intestine do not breakdown substances with opioid activity [86]. Others report they have been isolated in cerebral spinal fluid [87]. Hemmings reported over 30 years ago that gluten fragments from oral ingestion were observed in the brain [88]. Hypothetically, they can be absorbed by an inflamed ('leaky') gut, enter into the CNS, and interfere with normal brain function based on the theory that these peptide fragments act as endogenous opioids [4]. This opioid theory explains a potential pathophysiological mechanism behind the need for a casein-free/gluten-free diet [89], but it remains speculative at best.

A review by Lipkin and Honig investigates interactions between genes, microbes, toxins and other environmental agents that can compromise the neural circuitry [90]. It is based on the unproven hypothesis that certain substances absorbed through the intestines can affect the CNS. However, it remains difficult to decipher the effects of substances passing through the blood–brain barrier.

Despite this fact, celiac disease, an immune-mediated enteropathy, has a genetic link that is associated with impaired intestinal permeability and neurological consequences of not following a gluten-free diet: primarily depression, memory loss and confusion [91,92]. There appears to be an increased risk of developing celiac disease in individuals with schizophrenia [93]. Although multiple clinical trials with schizophrenic individuals on gluten-free diets show little improvement in psychiatric symptoms, Kalydjian *et al.*, in their review of the literature, suggest that a subset of schizophrenic patients do show "a drastic reduction, if not full intermission" on a gluten-free diet [93].

In any case, even if the theory of gliadomorphins and casomorphins cannot be shown to affect the CNS, it could be suggested that gluten and casein should be removed from the diet if lactose intolerance and gluten sensitivities are contributing to GI inflammation and related problems in children with ASD. Even if the hypersensitivity is to milk proteins, lactose intolerance may result from an inflamed, compromised intestine and as such may need to be limited. Ibrahim *et al.* has criticized the association of GI problems and autism, arguing that parents spend unnecessary time, energy and money on ineffective therapies based upon a gut–brain hypothesis [60].

Dysbiosis theory

Speculation continues as to whether or not autism can be linked to abnormalities in the bowel. Specifically, dysbiosis or bacterial imbalance in the bowel has been questioned as a possible problem linked to children with regressive-onset autism. This type of autism occurs in a third of cases of autistic disorder. The child progresses normally for 1–2 years and then loses previously developed skills. Sandler *et al.* studied such children (43–84 months) who exhibited autism within 2 months of developing chronic diarrhea from treatment with broad spectrum antibiotics [94]. One of the investigators on this study, Bolte, had a son that became autistic after being treated with antibiotics [95]. Bolte hypothesized that antibiotics might disrupt the normal intestinal flora, thus allowing the growth of neurotoxin-producing bacteria [96]. After Sandler *et al.* administered an antibiotic, vancomycin (500 mg/day for 8 weeks) to their subjects, eight out of ten (80%) autistic children showed improvement [94]. However, all but one deteriorated within approximately 2 weeks following antibiotic discontinuation. They suggested the possible gut flora–brain connection warrants further investigation.

GI dysfunction in other developmental delay disorders

Since GI disturbances, including celiac disease, are common in subsets of children with other developmentally delayed disorders, does an equivalent increased risk occur in children with autism?

Constipation

Mental retardation affects 1% of the US population and there is no debate that constipation and fecal impaction are common among these children [97]. Approximately 30% of children with autism have some degree of mental retardation. Are they at risk for the GI problems associated with mental retardation? Not only are constipation rates higher in children with autism [60], but it also affects children with Rett syndrome [98], Prader–Willi syndrome and Hunter disease [99].

Table 1. Estimated prevalence of celiac disease in patients among three developmental delayed conditions.

Study (year)	Condition	Estimated % with celiac disease	Ref.
Hilhorst *et al.* (1993)	Down syndrome	5–12	[104]
Bonamico *et al.* (2002) Gillett *et al.* (2000) Gravholt *et al.* (2005) Ivarssson *et al.* (1999)	Turner syndrome	4–8	[105] [106] [107] [108]
Gionnotti *et al.* (2001) Rujner *et al.* (2001)	Williams syndrome	8	[109] [110]

Other GI problems

In our previous publication we addressed the fact that other GI problems reported in genetically related mental disorders include possible functional megacolon in Rett syndrome, where 76% of these patients experience GI symptoms [100], gallbladder dysfunction (gallstones and cholecystectomy) in Rett syndrome [101] and gastroparesis in Prader–Willi syndrome [102]. Levy even stated that "Down syndrome is recognized as one of the most common predisposing conditions for a group of serious GI anomalies – tracheo–esophageal fistula, duodenal obstruction with or without pyloric stenosis, annular pancreas, imperforate anus and Hirschsprung's disease" [103]. He added that "intestinal anomalies can be found in many other genetic disorders, with recent evidence suggesting the presence of GI developmental regulatory genes on chromosome 13q."

Box 1. Gastrointestinal assessment checklist for a subset of children with autism and gastrointestinal dysfunction.

Assessment for gastrointestinal dysfunction

Symptoms

- Chronic constipation
- Diarrhea
- Vomiting:
  - Bilious emesis
  - Protracted vomiting
  - Cyclical vomiting
- Abnormal stools:
  - Pellets
  - Bulky
  - Unformed and mushy
  - Bloody
- Bloating
- Belching
- Reflux (GERD)
- Flatulence/gas
- Abdominal pain
- Encopresis (involuntary fecal soiling)
- Unexplained fever

Further evaluation

- Family history of inflammatory bowel disease or any autoimmune disease
- Inflammation of the GI tract
- Enteric nervous system abnormalities
- Disturbed sleep pattern
- Unexplained irritability
- Vocal behaviors – complains while pointing to abdomen, cries (or whines, groans or screams) for no reason and/or repeatedly clears throat
- Motor behaviors – grimacing, gritting teeth, wincing, applying pressure to abdominal area (leaning over furniture, lying abdomen down, pressing hands into or rubbing abdomen), grazing, pica, chewing clothes and/or straining to pass stools

GERD: Gastroesophageal reflux disease.
Adapted from [71,117]. © 2011 Amy Brown.

Celiac disease

In terms of celiac disease, The North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHN) states that at least three developmentally delayed conditions have higher risk rates for celiac disease than in the normal population (Table 1) [104–111]. Approximately 44% of cerebral palsy subjects also have high test results for serological celiac markers [112]. Celiac disease risk is also higher in children with Down syndrome. In fact, the 1999 Health Care Guidelines by the Down syndrome Medical Interest Group recommends an initial screening for celiac disease at the age of 2 years [113]. We suggest that screening be conducted not only in children with Down syndrome but also in all other developmentally delayed conditions, including ASD. These children are at a higher risk for celiac disease or gluten sensitivity and not testing them predisposes this minority to possible untreated intestinal pain that may trigger certain behavioral problems. Although not a developmental delayed condition, Type 1 diabetes mellitus is associated with a possible autoimmune etiology, and NASPGHN recognizes increased rates of celiac disease in this group. The 2009 American Diabetes Association Standards of Medical Care in Diabetes listed celiac disease rates in this group as 1–16% versus 0.3–1% in the general population. They recommended celiac disease screening in individuals with Type 1 diabetes [114]. Recent screening guidelines were published by the Federation of International Societies of Pediatric Gastroenterology, Hepatology, and Nutrition consensus report on celiac disease [115].

Expert commentary

Sufficient evidence exists to support the hypothesis that at least a subgroup of children diagnosed with ASD suffers from altered immune function and GI disturbance. Asperger himself suggested a half century ago in 1961 that there was a possible link to celiac disease in autism [116], and Kanner also made note of GI problems in children with autism [40]. A review in *Pediatrics* lists 11 studies showing the prevalence of GI symptoms in people with ASD is approximately 40% (range: 9–91%) [117].

In summary, higher rates of autoimmune conditions exist in ASD families and a subset of children with ASD appear to have higher rates of infections, allergies (including delayed-type food allergies), increased autoantibodies, antibodies to casein and gluten, colonoscopy observations of GI inflammation and ileal-lymphoid-nodular hyperplasia, and higher rates of food selectivity, constipation, gastroesophageal reflux disease and other GI symptoms. Insufficient studies have been conducted to determine whether higher rates of celiac disease occur in children with ASD; however, it also appears to affect up to approximately 10% of children afflicted with Down syndrome, Turner syndrome and Williams–Beuren syndrome.

It was the purpose of this narrative review to bring the autoimmune and immunodysregulatory–GI dysfunction seen in autistic disorder to the forefront. It is only a hypothesis, but we suggest that autism is just one of many autoimmune conditions linked to GI dysfunction that includes, but is not limited to, the disruption of the protective GI barrier (mucosal and tight junctions) resulting in symptoms that are currently not being sufficiently assessed

or treated in medical practice. Conversely, perhaps it is more likely that GI symptoms observed in autism patients may share a common basis of their autoimmune conditions associated with immunodysregulation. Is it possible that individuals genetically prone to an autoimmune condition are at an increased risk for GI dysfunction? It hints at, but does not prove, that many other autoimmune conditions may be subject to the same scenario. Such is the case for Down syndrome and many other development delayed conditions, including Crohn's disease, autoimmune thyroid disease, Type 1 diabetes mellitus, celiac disease and even AIDS, to name a few.

Down syndrome is mentioned because this is a known developmental delayed disorder that has a documented higher risk for celiac disease. These patients have significant immune disorders, one of which may be celiac disease. Our point is why could this not also be true for children with autism? Is it possible that they may also have an immune dysfunction like children with Down syndrome, thus increasing the risk of celiac disease and other GI immune-related problems? More importantly, this autoimmune–GI dysfunction connection in Down syndrome and now autism may reveal a link to other autoimmune conditions that need to be fully explored. We urge researchers to pursue this important topic that may provide clues relating not only to autistic disorder, but to all autoimmune conditions.

Five-year view

Future medical practice

Currently, a subset of children with GI dysfunction that may be related to an autoimmune disorder are neither being identified nor treated. These children, including those with autistic disorder, should not have to await confirmation of current theories explaining the pathophysiology of research findings if symptomatic treatment is available.

To remedy this problem, we support the recent recommendations published in *Pediatrics* proposing for the first time that ASD children be screened and treated for GI symptoms [71,117]. These authors state that children with ASDs can benefit from the evaluation of abdominal pain, chronic constipation and other GI symptoms. We provide a practical GI assessment checklist summarized in a table format (**Box 1**), derived from the extensive review by Buei *et al.* [117]. Perhaps this will help identify the subset of children with autism that have GI dysfunction. In addition, **Box 2** provides a nutritional assessment checklist to accompany the GI assessment. Ideally, positively screened patients should be referred to an allergist, gastroenterologist and or registered dietitian, where appropriate.

Future research

Specific suggestions for conducting research in the next 5 years within the possible subset of autistic children experiencing GI symptoms include:

- Identifying autism subgroups so that the diagnosis could eventually have pathophysiological differentiation with specific subdiagnosis criteria;
- Creating GI symptom criteria to screen for food sensitivities;
- Utilizing the GI symptom criterea to screen for specific subjects in any future GI-related autism studies, rather than indiscriminately selecting subjects from the greater pool of autistic children;
- Determining whether this GI symptom-prone group has higher rates for food sensitivities, food allergies, lactose intolerance, celiac disease and/or other GI enzyme deficiencies or autoimmunities;
- Determining whether the appropriate dietary therapy, such as an elimination diet (discussed in our previous paper [118]), or any other diet (not just a casein-free/gluten-free diet), is effective in alleviating GI and/or behavioral symptoms;

Box 2. Nutritional assessment for a subset of children with autism and gastrointestinal dysfunction.

Symptoms or testing

- If allergic disease present:
  - Allergist referral – for skin testing, measurement of allergen-specific IgE levels, elimination diets and food challenges
  - Gastroenterologist referral for laboratory testing and endoscopy, if indicated
- Food sensitivities:
  - Dairy
  - Wheat/Gluten
  - Others
- Test for lactose intolerance:
  - Strict lactose-elimination diet
  - Lactose breath test (difficult due to overnight fast and repeated breath tests over 3 h
- Test for celiac disease:
  - Total IgA level
  - Tissue transglutaminase IgA antibodies with or without endomysial IgA antibodies

Note: children on a gluten-free diet should be tested when gluten is reintroduced. Those staying on such a diet can be genetically tested for *HLA-DQ2* and *HLA-DQ8*

- Assess for nutritional deficiencies, especially:
  - Protein
  - Calcium
  - Vitamin D
- Monitor anthropometry and compare to growth charts [201]:
  - Height
  - Weight
  - Head circumference
- Diets†:
  - Refer to registered dietitian to prevent nutritional deficiencies
- Antibiotic and antifungal therapies not recommended
- Treatment to alter intestinal flora should not be started before an abnormal culture is obtained (duodenal aspirate or abnormal stool culture)

†Anecdotal reports that suggest restricted diets may ameliorate disorder symptoms have not been strongly supported or refuted by the scientific literature to date. The literature also does not fully address the possibility that a subgroup of individuals may respond to such diets.
Adapted from [71,17].

- Determining the prevalence of GI symptoms in all autoimmune conditions (partially listed in [17]), not just autism;
- Testing the effectiveness of an elimination diet in reducing GI symptoms in all autoimmune conditions coupled with GI symptoms that show evidence of immune reactivity to dietary proteins and or food intolerances.

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Key issues

- Autism patient families demonstrate a higher rate of autoimmune disorders such as Type 1 diabetes, rheumatoid arthritis, hypothyroidism and systemic lupus erythematosis.
- In 1943, Kanner, who originated the 'early infantile autism' diagnosis, documented symptoms of higher rates of infections. Research now reports that autistic children have higher rates of otitis media involving repeated rounds of antibiotics, allergies, delayed type food allergies, and pediatrician and hospital visits.
- Reviews now suggest that some autism polymorphisms may relate to a congenital immune deficiency and/or faulty autoimmune mechanisms. This is based on reports of altered autoantibody production, other immune imbalances and not unexpectedly, higher rates of infections and food allergies in children with autism spectrum disorders (ASD).
- Food allergies may result in gastrointestinal (GI) symptoms. A *Pediatrics* review reported that the prevalence of GI symptoms in children with ASD ranged widely from 9 to 91%. This was based on 11 studies that together give an average incidence of 40%.
- In one study, nine out of 21 children (43%; 4–16 years of age) with ASD had increased intestinal permeability. Researchers do not suggest that GI disease is the cause of autism, nor that intestinal permeability is the cause of GI problems in certain autoimmune conditions. Rather, intestinal permeability is one step in a long continuum of steps leading up to GI dysfunction and other related symptoms.
- Since GI disturbances, including celiac disease, are common in subsets of children with other developmentally delayed disorders, does an equivalent increased risk occur in children with autism?
- Sufficient evidence exists to support the hypothesis that at least a subgroup of children diagnosed with ASD suffer from altered immune function and GI disturbance.
- This article supports the recent recommendations in *Pediatrics* that ASD children be evaluated and treated for GI symptoms. We encourage the use of **Boxes 1 & 2** for this process.
- Affected children should not have to await confirmation of current theories explaining the pathophysiology of research findings if symptomatic treatment is available.

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