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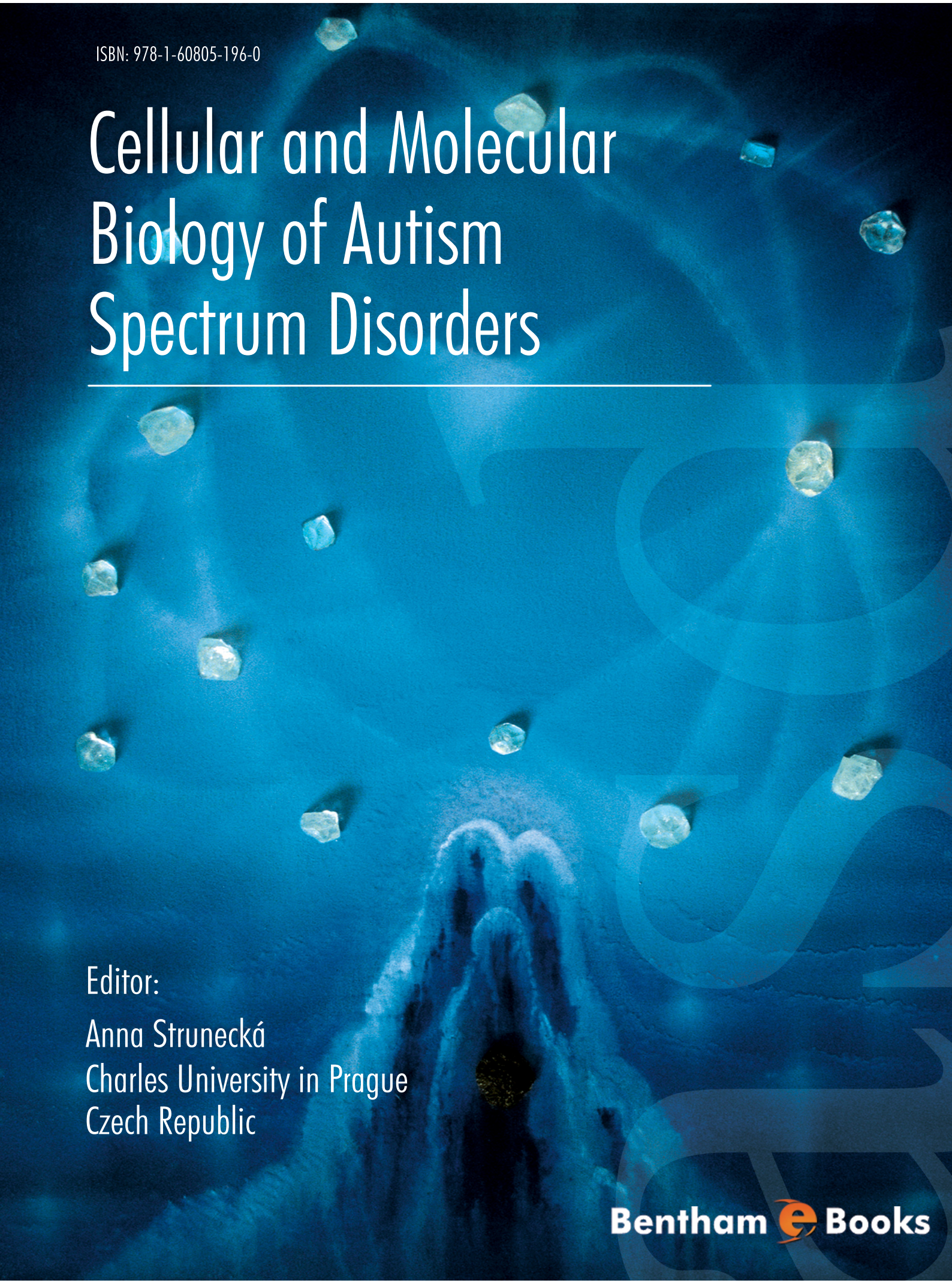
Cellular and Molecular Biology of Autism Spectrum Disorders



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CELLULAR AND MOLECULAR BIOLOGY OF AUTISM SPECTRUM DISORDERS

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Dedication

To scientists who search for the origin of autism.

To physicians who explore how to best help those suffering from autism.

To parents, relatives and friends who care for people with autism every day.

To all of us who desire to understand what autism means.

Cover Design

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FOREWORD

The history of the biomedical movement in autism treatment began in the 1960's when Dr. Bernard Rimland, founder of the Autism Research Institute (ARI), took a monumental step forward by declaring that autism was due to a physiological abnormality rather than a result of poor nurturing by uncaring parents. Soon after his 1964 book, *Infantile Autism*, was published, he was besieged with letters and telephone calls from parents worldwide who praised his writings and shared their own personal journeys with autism.

Shortly after the publication of this seminal book, Dr. Rimland was astonished at the number of parents who reported observing significant improvements in their children soon after giving them a nutritional supplement. He conducted several formal and informal studies, and concluded that vitamin B6 with magnesium might help up to 50% of the autism population. To date, there are 11 placebo-controlled studies supporting the efficacy of vitamin B6 and magnesium as a treatment for autism. Another biomedical-related intervention, reported by Dr. Rimland in the 1970s, was the importance of restricted--and healthy diets.

Over the years, parents as well as clinicians continued to write to Dr. Rimland about their experiences; in turn, ARI would share this information with research scientists and clinicians around the world.

The year 1995 was a turning point in the biomedical field; Dr. Rimland, along with two of his close colleagues, Drs. Sidney Baker and Jon Pangborn, convened the first international think tank on autism. Over 30 researchers and clinicians were invited to meet for two-and-a-half days. Toward the end of the meeting, they agreed on the importance of investigating gastrointestinal (GI) and immune system problems more deeply, to better understand and treat individuals on the autism spectrum.

2010 has also been an important one for the biomedical field. The American Academy of Pediatrics' journal, *Pediatrics*, published a consensus report on the state-of-the-art research on GI problems associated with autism. A few months later, a large-scale multi-center survey involving 1,185 children and teenagers on the autism spectrum showed that nearly half (45%) had one or more forms of GI problem.

Viewed from a broader perspective regarding the treatment of autism, one of the problems in the field is a clash with clinicians and researchers who favor other effective forms of treatment, such as Applied Behavior Analysis (ABA) and sensory interventions. The viewpoint taken by many people in the biomedical field, including those at ARI, is that many, but not all, individuals on the autism spectrum suffer from some type of medical problem, such as GI and/or immune system dysfunction, and these problems can lead to discomfort or pain, cause sensory dysfunction, impede executive functioning, and more. Once the person's health improves, many of their sensory problems are reduced or are eliminated, and they will be primed to attend, and thus to learn in an educational setting.

Clinicians and researchers worldwide are striving for a global standard of care for individuals on the autism spectrum. We need to recognize their various needs or problems--medical, sensory, and behavioral--but we also need to be cognizant of individual differences in this population. Through networking, communicating, and discouraging politically-oriented science, it will be made possible for individuals on the autism spectrum to reach their true potential, and their quality of life will improve significantly.

Stephen M. Edelson,
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PREFACE

Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders characterized by abnormalities in social interaction, language function and communication, and abnormalities in the realm of behavior. Over the past several decades the incidence of ASD has increased dramatically, with much of the increase not being explained by improved diagnosis. The etiology of ASD remains an unsolved puzzle to scientists, physicians, pediatricians, psychiatrists, and pharmacologists. Of great concern is that no central mechanism has been proposed to explain the various clinical presentations of the ASD and no evidence-based therapy has been offered. The advantage of this eBook is to discuss the state of knowledge regarding the pathophysiology, cellular and molecular biology of these disorders.

A great number of biochemical and pathological changes have characterized ASD, adding confusion to discovering a common etiology. A recent review of the genetic links to ASD found that the most common genes suspected were operate glutamate receptors (GluRs), either ionic or metabotropic. A considerable amount of evidence suggests a role for a dysfunctional immune system in the ASD. The crosstalk between GluRs and cytokine receptors leads to neurodegeneration, abnormal neuronal migration patterns, seizure generation, and dysfunctional brain connectivity. When combined with the finding of elevated glutamate in a number of autistic children, this indicates a possible hyperactivity of GluRs in those at greatest risk.

Our eBook explains, for the first time, the central role of immunoexcitotoxicity in the etiopathogenesis of the broad spectrum of autistic disorders. Based on our hypothesis of immunoexcitotoxity, we integrate various findings in ASD with this hypothesis. A careful review of known environmental and pathological links to ASD indicates that most, if not all, are connected to the immunoexcitotoxic process. Our eBook also offers treatment proposals that address each of these mechanisms. It explains how previous, often successful treatment methods, may indeed operate through the immunoexcitotoxic mechanism.

The tremendous research of individuals with ASD shows most explicitly that ASD is neither a disease of one gene, neurotransmitter or hormone, nor a disease of a single isolated second messenger disturbance. The enormous increase of autism during last decade inevitably requires an integrative approach, which brings together not only specialized scientific knowledge, but also knowledge about the homeostatic mechanisms of the whole human being. We simultaneously realize that the living system does not behave as a static jigsaw puzzle. The behavior of a whole cannot be predicted by knowing the separated parts. We hope that the integration of specialized knowledge about molecular and cellular mechanisms could lead to understanding why new generations suffer with an epidemic of autism.

Our eBook reviews the studies of scientists from the broad area of neurosciences and neuropharmacology, cognitive and affective developmental neuroscience; researchers from immunology, pathophysiology, and developmental biology; researchers from the developmental psychopathology and applied behavioral analysis; practicing physicians, pediatricians, psychiatrists, and psychologists; but also parents and care-givers, who are in daily contacts with children and adults with autism.

Author’s thanks belong to Hana Kruzikova for her excellent cooperation in preparing diagrams and figures and to PhDr. Ing. Zdenek Hajny, PhD., for providing his picture for the cover.

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CHAPTER 6

Gastrointestinal Disorders and Autism Spectrum Disorders: A Causal Link or a Secondary Consequence?

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Abstract: Growing evidence confirms that up to 95% of autistic children suffer with the dysfunctions of the gastrointestinal (GI) system. We discuss the cellular and molecular mechanisms underlying these disturbances. Some researchers, physicians, and health care professionals suggest that beneficial effects of dietary intervention on behavior and cognition of some autistic children indicate a functional relationship between the GI tract (GIT) and the CNS pathology of ASD. A possible genetic cause for the association of autism and GI disease is discussed. GI disorders are not included in diagnostic criteria for ASD. Clinical and practical experiences provide the support for association between inflammatory bowel disease and ASD.

INTRODUCTION

Parents of autistic children have much experience from daily life with chronic diarrhea, bloating, abdominal pain, distension, and abnormal stool consistency. Moreover, children with ASD have often unusual feeding patterns and narrow range of preferred dishes [1]. Although not included in the diagnostic criteria, there have been many reports describing GI symptoms in 9 to 84% or more of children with ASD [2-10]. Clinical studies have confirmed that the most common GI symptoms in patients with ASD are constipation, diarrhea, and abdominal distension. Chronic inflammation of the GIT, food intolerance, and recurrent GI symptoms are often recorded by the general practitioners [11,12].

Several clinicians and many parents admit that a treatment of digestive problems may have positive effects on autistic behavior [4]. GI disorders can present as non-GI problems. For example, Horvath and Perman [4] reported disturbed sleep and nighttime awakening for 52 percent of children with ASD who had GI symptoms in comparison with seven percent of age-matched healthy siblings. Some researchers, physicians, and health care professionals suggest that a beneficial effect of dietary intervention on behavior and cognition of some autistic children indicates a functional relationship between the alimentary tract and the CNS pathology of ASD. Increased gut-blood-brain barrier (BBB) permeability might be involved in this link. Altered behaviors are often linked to abdominal pain or discomfort in children with ASD [13-15].

Recently, a multidisciplinary panel reviewed the medical literature with the aim of generating evidence-based recommendations for diagnostic evaluation and management of GI problems in ASD patient population [16]. On May 29 –30, 2008, the multidisciplinary panel convened in Boston, Massachusetts, to review and discuss GI aspects of ASD. Meeting participants were part of a larger group that was organized to develop recommendations for the evaluation and management of GI disorders for individuals with ASD, as well as for future research directions. Working groups comprised 28 experts in child psychiatry, developmental pediatrics, epidemiology, medical genetics, immunology, nursing, pediatric allergy, pediatric gastroenterology, pediatric pain, pediatric neurology, pediatric nutrition, and psychology. A literature search on Medline was conducted to identify relevant articles by using the key words “gastrointestinal disease” and “autism.” A consensus report was released on January 2010 [16]. However, the expert panel reached consensus on 23 statements. The panel admits that:

- GI disorders and associated symptoms are commonly reported in individuals with ASD,
- but key issues such as the prevalence and best treatment of these conditions are incompletely understood.

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- Because of the absence, in general, of high-quality clinical research data, evidence-based recommendations are not possible at the present time.
- Individuals with ASD deserve the same thoroughness and standard of care in the diagnostic workup and treatment of GI concerns as should occur for patients without ASD.

Children with ASD can benefit from adaptation of general pediatric guidelines for the diagnostic evaluation of abdominal pain, chronic constipation, and gastroesophageal reflux disease [17]. These guidelines help health care providers determine when GI symptoms are self-limited and when evaluation beyond a thorough medical history and physical examination should be considered. Children with ASD who have GI disorders may present with behavioral manifestations. Diagnostic and treatment recommendations for the general pediatric population are useful to consider until the development of evidence-based guidelines specifically for patients with ASD.

Many doctors and scientists have ignored the fact that up to 95 % of autistic children have intestinal problems, such as altered bowel function and abdominal distension [14]. There appeared some studies in a literature search of Medline, which document that there were no reports of inflammatory bowel disease or autism over the study period [18]. Kuddo and Nelson found that the frequency of GI symptoms observed in population-based samples of autistic children indicate that GI problems are not nearly as common in children with autism as reports from pediatric gastroenterology clinics suggest [19]. The recent study of Ibrahim and coworkers examined 124 adult patients with ASD and concluded that no significant associations were found between autism case status and overall incidence of GI symptoms or any other GI symptom category [20]. These authors suggest that a neurobehavioral rather than a primary organic GI etiology may account for the higher incidence of these GI symptoms in children with autism.

The first steps to understanding of association of GI dysfunction with ASD provide recent genetic studies, which demonstrate how disruption of a candidate gene *MET* contributes to ASD risk [21-24]. The MET (mesenchymal epithelial transition factor) receptor tyrosine kinase (RTK) participates not only in development of the cerebral cortex and cerebellum, both of which may be altered in ASD (see Chapter 2), but also contributes to GI and immune function, disruptions of which co-occur in some patients with ASD. GI disorders of patients with ASD have been subjects of numerous discussions and great surrounding controversies. Some authors suggested that GI problems have more commonly been linked to regressive forms of ASD, characterized by loss of previously acquired skills and late onset of behavioral anomalies, not observed in the first year of life [2,25-28]. A link has been postulated between measles-mumps-rubella (MMR) vaccine and a form of autism that is a combination of developmental regression and GI symptoms that occur shortly after immunization.

In contrast, no association between developmental regression and GI symptoms has been repeatedly reported [6,29-32]. No significant difference was found in rates of bowel problems or regression in children who received the MMR vaccine during the 20 years from 1979 [33]; strong evidence against association of autism with MMR exposure was provided by Hornig et al [34]. Although great controversy has plagued those who suggested a link between the MMR vaccine and autism, history alone suggests that more research is needed to determine if there is a unique GI lesion in children with PDD [9]. The debate regarding the potential trigger of GI pathology and regressive autism is on going, exceeding the scope of a literature search on Medline.

The aim of this chapter is to review and integrate the scientific studies with clinical and practical experiences and with the new concept that the central mechanism of ASD is immunoexcitotoxicity [12,35] (see Chapter 4). The ultimate goals are to understand the etiology of ASD and to search for the best treatment of these conditions in children and adults with ASD to improve the quality of their life. Several questions thus arise:

- Has GI pathophysiology a causal role in the etiology of ASD?
- Is GI pathophysiology in ASD contributory to autism symptoms?
- Is there a link between GI disorders and autistic behavior?
- Can therapy of GI disorders lead to amelioration of GI symptoms?

GI ABNORMALITIES IN CHILDREN WITH ASD

The history of association of autism with disturbances of the gut functions is seen in an excellent review by Gilger [9]. He reminds us that several authorities in autism research suggested a possible link to gut dysfunction and

neuropsychiatric dysfunction in autistic patients since sixties. However, the first more detailed studies investigating GI anomalies in autistic children were reported by Horvath *et al.* [36] and Wakefield *et al.* [2,37]. Horvath with coworkers evaluated the structure and function of the upper GIT in a group of patients with autism who had GI symptoms. Thirty six children (mean age 5.7 ± 2 years) underwent upper GI endoscopy with biopsies, intestinal and pancreatic enzyme analyses, and bacterial and fungal cultures. The most frequent GI complaints were chronic diarrhea, gaseousness, and abdominal discomfort and distension. Histological examination in these 36 children revealed grade I or II reflux esophagitis in 25 (69.4 %), chronic gastritis in 15 (42 %), and chronic duodenitis in 24 (66.6 %). Low intestinal carbohydrate digestive enzyme activity was reported in 21 children (58.3 %), although there was no abnormality found in pancreatic function. Horvath with coworkers suggested that unrecognized GI disorders, especially reflux esophagitis and disaccharide malabsorption, may contribute to the behavioral problems of the non-verbal autistic patients. High prevalence of histological abnormalities in the esophagus, stomach, small intestine and colon, and intestinal permeability were reported in later study [4,5].

In 1998 Wakefield with 13 coworkers published an article describing an ileal lymphoid-nodular hyperplasia (LNH) and non-specific colitis ("autistic colitis") found in nine of the twelve examined children. Colitis with ileal LNH in children with regressive autism has been repeatedly described in subsequent studies [25,27,37-39].

The GI disorders reported in ASD include: inflammation in both the upper and lower intestinal tract (esophagitis, gastritis, duodenitis, enterocolitis) with or without autoimmunity, LNH, increased intestinal permeability, low activities of disaccharidase enzymes, impairment of detoxification, dysbiosis with bacterial overgrowth and food intolerance in many children with ASD [2-4,6-10]. Chronic inflammation of the GIT, food intolerance, and recurrent GI symptoms are frequently recorded by the general practitioners [11]. Other GI abnormalities that have been described for individuals with ASD include gastroesophageal reflux disease, abdominal bloating, and disaccharidase deficiencies, as well as pathologic findings such as inflammation of the GIT and abnormalities of the enteric nervous system. Frequent patient and/or parent complaints have included chronic diarrhea, bloating, abdominal pain, distension, and abnormal stool consistency.

Recently, Russo and Andrews [41] analyzed the complete medical history records of the Autistic Genetic Resource Exchange (AGRE), a DNA repository and family registry sponsored by Autism Speaks, including contributing family members who have had extensive evaluations by a variety of pediatricians, psychiatrists, and other neurodevelopmental specialists. The diagnosis of autism for all patients was made using the standard ADI-R algorithm. The analysis of 692 children (mean age 9.1 ± 5.1 years) with autism and 187 non autistic siblings (mean age 10.5 ± □6.6 years) shows that autistic children compared to non-autistic sibling controls have significantly higher overall GI disease (43 % vs. 12 %), chronic diarrhea (26 % vs. 13 %), and constipation (33 % vs. 13 %). According to expert panel report [16] the most common GI symptoms and signs reported for persons with ASD are chronic constipation, abdominal pain with or without diarrhea, and encopresis as a consequence of constipation.

Are Children with Autism More Likely to Have a History of GI Disorders than Children without Autism?

Black *et al.* [11] evaluated records from UK General Practice Research Database and found that 9 of 96 (9 %) children with a diagnosis of autism (cases) and 41 of 449 (9 %) children without autism had a history of GI disorders before the index date (the date of first recorded diagnosis of autism in the cases and the same date for controls). The authors thus concluded that no evidence was found that children with autism were more likely than children without autism to have had defined GI disorders at any time before their diagnosis of autism. Their study included only obvious GI disease and symptomatology and recognized that they might miss more subtle symptoms of GI disease. No association between chronic GI symptoms and a history of developmental regression was found in several studies [18, 30, 42]. Another study site was a clinic specializing in ASD in a large pediatric medical center serving a 10 county area in the mid-western USA. In a sample of 137 children, age 24-96 months, classified as having autism or ASD by the Autism Diagnostic Observation Schedule-Generic, 24 percent had a history of at least one chronic GI symptom. The most common symptom was diarrhea, which occurred in 17 percent of patients. However, authors concluded that no association between chronic GI symptoms and a history of developmental regression has been found [6].

On the other hand, in the first study of Wakefield *et al.* [2] 12 children (mean age 6 years, range 3-10, 11 boys) were referred to a pediatric gastroenterology unit with a history of normal development followed by loss of acquired

skills, including language, together with diarrhea and abdominal pain. Parents associated an onset of behavioral symptoms with MMR vaccination in eight of the twelve children, with measles infection in one child, and otitis media in another. Symptoms appeared one day to two months after immunization. The hypothesis put forward by the authors is that MMR vaccine causes inflammation or dysfunction of the intestine, increasing the absorption of non-permeable peptides, which in turn can cause serious developmental disorders. The Wakefield's group thus identified associated GI disease and developmental regression in a group of previously normal children, which was generally associated in time with possible environmental triggers. Colitis with ileal LNH in children with regressive autism has been repeatedly described in subsequent studies [25,27]. The GI symptoms had developed coincident with the onset of autistic behavior, according to parents. Wakefield postulated the hypothesis that there exists a subset of children who are vulnerable to developing a particular form of regressive autism following previously normal development, in combination with a novel form of inflammatory bowel disease. Onset may occur over weeks or sometimes months, and is triggered by exposure to a measles-containing vaccine, predominantly the MMR. This exposure leads to long term infection with measles virus within key sites, including the intestine where it causes inflammation. This hypothesis has been confirmed by subsequent studies of Wakefield ´s group with 60 and 148 autistic children with the regressive autism [38, 39]. In contrast, several authors found no evidence to support a distinct syndrome of MMR-induced autism [32].

Inflammation of GI System in Patients with ASD

We have explained in previous chapters that chronic inflammation plays a significant role in ASD. The sources of chronic stimulation are less clearly defined. Wakefield with coworkers reported the findings of LNH as the sign of inflammation and concluded that a new variant of inflammatory bowel disease "autistic enterocolitis" is present in the group of children with developmental disorders [2,38,39].

Lymph nodules are encapsulated bodies lying within the submucosa of the intestinal wall. These lymph nodules contain lymphocytes and neutrophils. The fluid absorbed from the intestinal lumen by the action of the absorptive epithelial cells is filtered through the lymph nodes. Here, antibodies are formed [13]. The study of Wakefield *et al.* [38] found LNH in 54 of 58 children with developmental disorders. Scores of frequency and severity of inflammation were significantly greater in affected children, compared with controls. In this trial, ileal LNH was present in 93 % of affected children versus 14.3 % of controls and chronic colitis in 88 % of affected children versus 4.5 % of controls. Active inflammation of the ileum (ileitis) was observed in 8 % and chronic inflammation of the colon (colitis) was seen in 88 % of affected children.

In a comment on the Wakefield paper, Sabra *et al.* [43] reported identical pathology (LNH) in the terminal ileum of two children patients diagnosed with pervasive disorders. Immunohistochemistry confirmed a distinct lymphocytic colitis in ASD in which the epithelium appears particularly affected. This is consistent with increasing evidence for gut epithelial dysfunction in autism [25]. In the later study, Wakefield and coworkers [39] investigated 148 consecutive children with ASD (median age 6 years; range 2-16; 127 male) with GI symptoms by ileocolonoscopy. They found that the prevalence of LNH was significantly greater in ASD children compared with controls: being present in the ileum 129/144 (90 %) vs. 8/27 (30 %), and colon 88/148 (59 %) vs. 7/30 (23 %), whether or not controls had co-existent colonic inflammation. The severity of ileal LNH was significantly greater in ASD children compared with controls.

With respect to the upper GIT, Horvath *et al.* [36] investigated 36 autistic children complaining of abdominal pain, bloating or chronic diarrhea by gastroscopy. The most common histological finding was reflux esophagitis (69.4 %), while 41.7 % had chronic gastritis and 66.7 % had chronic duodenitis in the absence of *Haemophilus pylori* infection. The number of Paneth cells in autistic children was also noted to be significantly elevated compared with neurotypical controls. Torrente *et al.* [26,28] found that 11 of the 25 autistic children had a focally enhanced gastritis, while two had mild diffuse gastritis. Immunohistochemistry results demonstrated the pattern of lymphocyte infiltration was most similar to Crohn's disease, with the exception of a striking predominance of CD8-positive over CD4-positive cells and a marked increase in intraepithelial lymphocytes. Another highly specific finding among autistic children was a dense, subepithelial basement membrane immunoglobulin G deposition, which was absent in the other subgroups.

It is important to note that the Horvath *et al.* [36] and Torrente *et al.* [26,28] studies describe inflammation in the upper GIT of autistic children, whereas Wakefield and coworkers observed inflammation in the ileum and colon.

The results of these different studies taken together suggest that inflammation of GIT may accompany ASD. Wakefield *et al.* [2,38,39] suggested a relationship between severe intestinal inflammation secondary to MMR vaccines and ASD. This idea evokes a strong controversy, which is going beyond the scope of scientific interests. Nevertheless, some studies have linked the live measles virus from MMR vaccine to the inflamed GIT. It has not been excluded that a weakened immune system of some children is unable to produce protective antibodies even against an inactivated live virus. Then the live attenuated virus persists, producing low-grade inflammation – in both the gut and the brain [14]. This does not mean that all children who are vaccinated will develop ASD.

In contrast, some evidence against association of autism with persistent measles virus RNA in the GIT or MMR exposure was provided by the study of Hornig *et al.* [34]. This study evaluated the measles virus RNA in ileal and cecal tissues from 25 children with autism and GI disturbances and 13 children with GI disturbances alone (controls). No differences were found between case and control groups in the presence of measles virus RNA in ileum and cecum. Hyman [14] suggests that this finding does not rule out the possibility that the virus did its harm in a “Hit-Run“ fashion. In the "Hit-Run" hypothesis, virus infects the periphery but never enters the CNS. The virus sets up an abnormal immunologic milieu for subsequent autoimmunity. Yet, there is compelling evidence that the measles virus does enter the brain and frequently becomes persistent. Katayama *et al.* demonstrated that measles virus commonly persists in the human brain without causing apparent clinical symptoms, probably due to decreased virus replication [44].

Based on their long-term investigations, Wakefield and coworkers concluded that:

- Ileo-colonic LNH is a characteristic pathological finding in children with ASD and GI symptoms.
- LNH is associated with mucosal inflammation.
- Inflammation is much more severe in autistic children compared to children without autism.

It has been mentioned that several authors found no evidence to support a distinct syndrome of MMR-induced autism or of "autistic enterocolitis." The expert panel [16] concluded that:

- At present, there are inadequate data to establish a causal role for intestinal inflammation.

Many doctors and scientists suggest that autistic children are more susceptible to GI inflammation triggered by certain foods, namely gluten and casein. Immune reactivity to dietary proteins may be associated with GI inflammation in ASD children that may be partly associated with aberrant innate immune response against endotoxin, a product of the gut bacteria [45].

Gastrointestinal Tract and Immune System

It has been generally known that the GIT is the largest immune organ in the body, containing up to 80 % of immune globulins-producing cells in the body. In children with ASD, immunohistochemistry and flow-cytometry studies have consistently shown marked panenteric infiltration of lymphocytes and eosinophils in the gut mucosa [25-28]. Torrente *et al.* suggested an autoimmune component to the inflammatory response – co-localized deposition of IgG and complement C1q on the surface epithelium of the GIT [26,28]. These studies suggest an underlying chronic inflammatory process in some individuals with ASD and co-occurring GI disturbances, characterized by LNH, enterocolitis, and mucosal infiltration by immune cells along the length of the GIT. According to Consensus of expert panel, these findings should be considered preliminary and will require confirmation [16].

Some data provide further evidence of a panenteric mucosal immunopathology in children with regressive autism that is apparently distinct from other inflammatory bowel diseases [27,46,47]. A more recent study found that peripheral blood lymphocytes as well as mucosal CD3 + TNF-α and CD3 + IFN-γ cytokine response were significantly increased in children with ASD as compared to non-inflamed control children. The critical difference between children with Crohn´s disease and those with ASD was that in the latter, peripheral and mucosal IL-10 responses were markedly lower. This indicated not only a GI autoimmune reaction in autistic children, but a suppression of the cytokine known to regulate immune termination, IL-10.

Evidence suggests that ASD may be accompanied by aberrant (inflammatory) innate immune responses. This may predispose ASD children to sensitization to common dietary proteins, leading to GI inflammation and aggravation of some behavioral symptoms. Jyonouchi with coworkers [48,49] measured IFN-γ, IL-5, and TNF-α production against gliadin, cow's milk protein, and soy by peripheral blood mononuclear cells (PBMCs) from ASD and control children. PBMCs obtained from ASD children with GI symptoms produced more TNF-α/IL-12 than those obtained from control subjects with CMP. They also produced more TNF-α with gliadin. A high prevalence of elevated TNF-α/IL-12 production by GI (+) ASD PBMCs indicates a role of non-allergic food hypersensitivity (NFH) in GI symptoms observed in children with ASD.

The Role of Gut Microflora in the Pathogenesis of GI Disorders in ASD

The human endogenous intestinal microflora is immensely diverse ecosystem. It has important role in regulating epithelial development and instructing innate immunity. Repeated use of antibiotic therapy may disrupt the complex microbial ecosystem and contribute to more favorable colonization by toxin-producing species [50]. Experience from multiple courses of antibiotic therapy is common in children with ASD. Many parents of children with regressive autism have noted antecedent antibiotic exposure followed by chronic diarrhea. Sandler *et al.* [51] recruited 11 children with regressive-onset autism for an intervention trial using a minimally absorbed oral antibiotic vancomycin. Entry criteria included antecedent broad-spectrum antimicrobial exposure followed by chronic persistent diarrhea. Short-term improvement was noted in 8 of 10 children studied. Unfortunately, these gains had largely waned at follow-up. Sandler concluded that although the protocol used is not suggested as useful therapy, these results indicate that a possible gut flora-brain connection warrants further investigation.

Statement number 19 from Consensus Report postulates that the role of gut microflora in the pathogenesis of GI disorders in individuals with ASD is not well understood [16]. Moreover, clinicians should obtain an abnormal culture result from a duodenal aspirate or abnormal stool culture before starting any treatment designed to alter intestinal flora. The experts noted that empirical antibiotic and antifungal therapy in patients with ASD is not recommended.

Finegold with coworkers suggested that some cases of regressive autism may involve abnormal flora [52]. Fecal flora of children with regressive autism was compared with that of control children, and clostridial counts were higher. The number of clostridial species found in the stools of children with autism was greater than in the stools of control children. Children with autism had nine species of *Clostridium* not found in controls, whereas controls yielded only three species not found in children with autism. In all, there were 25 different clostridial species found. In gastric and duodenal specimens, the most striking finding was total absence of non-spore-forming anaerobes and microaerophilic bacteria from control children and significant numbers of such bacteria from children with autism [52].

Several other studies demonstrate significant alterations in the upper and lower intestinal flora of children with ASD. Autistic children have been shown to have higher counts and more species of clostridia than age- and sex-matched controls [53-55]. Parracho *et al.* [55] studied the fecal flora of patients with ASD and compared them with those of two control groups (healthy siblings and unrelated healthy children). They found that the fecal flora of ASD patients contained a higher incidence of the *Clostridium histolyticum* group (*Clostridium* clusters I and II) of bacteria than that of healthy children. However, the non-autistic sibling group had an intermediate level of the *C. histolyticum* group, which was not significantly different from either of the other subject groups. Members of the *C. histolyticum* group are recognized toxin-producers and may contribute towards gut dysfunction, with their metabolic products also exerting systemic effects. Parracho with coworkers thus suggests that strategies to reduce clostridial population levels harbored by ASD patients or to improve their gut microflora profile through dietary modulation may help to alleviate gut disorders common in such patients.

In a number of studies, reaction to commonly found colon bacterial organism are seen to occur. The presence of β-1,5- glucan in the cell wall of the bacteria and yeast appear to be the most powerful immune component. The most common aerobic bacteria found in healthy individuals is *Escherichia coli* and it accounts for 90-95 % of all the aerobic bacteria. In Bioscreen's autistic study the average amount of *E. coli* was found to be quite low at approximately 56 % compared to the normal. In about 22 % of the autistic children the amount of *E. coli w*as actually less than 10 %, which is quite an incredible finding [56]. The second most common aerobe is *Enterococcus,*

although it is a lot less common than *E. coli,* at an average of five percent. There were also abnormal elevations in the amount of *Enterococcus* found in the feces of autistic children. This was found to be as high as 40 % in autistic children compared to the average of five percent in healthy individuals.

Many children afflicted with autism have had frequent ear infections as young children and have taken large amounts of antibiotics. There have been anecdotal reports of the onset of autism following broad-spectrum antibiotics, suggesting that disruption of the indigenous flora may lead to colonization by neurotoxin-producing bacteria and the overgrowth of yeast *Candida* [8].

Candida infections are often seen in children with ASD and have often been reported as being involved in pathology of ASD. Some clinicians believe that autistic symptoms are made worse by the overgrowth of *Candida albicans.* The role of candida is however still controversial. If it is present in the gut, it will undoubtedly affect the gut wall and increase permeability. It may also act as a source of strong chronic immunologic reactivity, especially if it penetrates the gut wall. When the yeast multiplies, it releases toxins in the body; and these toxins are known to impair the CNS and the immune system. The "leaky gut" theory of autism implies that treating yeast overgrowth should help the GIT to return toward normal and autistic symptoms to improve. Other possible contributors to candida overgrowth are hormonal treatments, immuno-suppressant drug therapy, exposure to herpes, chicken pox, or other "chronic" viruses or exposure to chemicals that might upset the immune system [57]. Thrush, the white yeast infection of the mouth and tongue, which is common in infants, is another well-known example of candida overgrowth.

> *"I am fairly well convinced that there is a connection and that perhaps 5% to 10% of autistic children—those given many courses of antibiotics, or born with thrush or afflicted with thrush soon after birth—will improve when properly treated for candida. However, there is no consensus among physicians on the candida/autism linkage. Meyer noticed that thrush seemed to be mentioned unusually often in the letters and questionnaires sent to us by parents. Treatment for Candida albicans infrequently results in a cure for autism. However, if the person is suffering from this problem, his/her health and behavior should improve following the therapy." Bernard Rimland [57].*

Increased Intestinal Permeability in Children with Autism

Leaky gut syndrome is an increase in permeability of the intestinal mucosa to luminal macromolecules, antigens and toxins associated with inflammatory degenerative and/or atrophic mucosal damage. Increased permeability has been cited as having a key role in various hypotheses regarding the biology of ASD, including excess opiate activity, diminished peptidase activity, and immune dysfunction. D'Eufemia *et al.* determined the occurrence of gut mucosal damage using the intestinal permeability test in 21 autistic children who had no clinical and laboratory findings consistent with known intestinal disorders [58]. An altered intestinal permeability was found in nine of the 21 (43%) autistic patients, but in none of the 40 controls. Compared to the controls, these nine patients showed a similar mean mannitol recovery, but a significantly higher mean lactulose recovery. These authors thus speculated that an altered intestinal permeability could represent a possible mechanism for the increased passage through the gut mucosa of peptides derived from foods with subsequent behavioral abnormalities.

However, the statement number five of Consensus Report postulates that the evidence for abnormal GI permeability in individuals with ASD is limited. Prospective studies should be performed to determine the role of abnormal permeability in neuropsychiatric manifestations of ASD [16].

Tight junctions represent the major barrier within the paracellular pathway between intestinal epithelial cells. Disruption of tight junctions leads to the increased intestinal permeability and is implicated in the pathogenesis of several acute and chronic pediatric disease entities that are likely to have their origin during infancy [59].

The Issue of Gluten and Casein in ASD

It is worth noting that Kanner in his paper describing autism in 1943 also detailed various somatic features as being present including GI disturbance and feeding problems. The original work of Hans Asperger from 1961 following his description of Asperger syndrome talked about a possible connection between this syndrome and problems with

foods containing gluten. In 1979 Panksepp described a neurochemical theory of autism proposing the incomplete breakdown and excessive absorption of dietary food. Over the years, these thoughts have been forgotten or ignored [9,15]. The recent definitions of ASD refer exclusively to irregularities in neuropsychological functioning.

It has been known for years that peptides from gluten and casein affect certain ASD children as to their behavior and overall cognitive function. Diet is widely known to affect both physical and mental health. It is not the topic of this chapter to deal with diets, but medicine is often based on the long-time experience. The collective experience of parents and some clinicians and care-givers supports the view that gluten-free (GF) or casein-free (CF) diet or combination of both (GFCF) has been used in autistic children with success. Parental reports detailing experience of removing gluten and/or casein from the diet of their children with ASD started to emerge in the 1980s. The exact way gluten and casein affect the body is not known. In this part, we will focus on the possible mechanisms explaining how gluten and casein might affect the course of autistic symptoms and we will mention the comparison with coeliac disease. However, The Consensus Report postulates that available research data do not support the use of a CF diet, a GF diet, or combined GFCF diet as a primary treatment for individuals with ASD.

Why is Gluten Harmful for Individuals with ASD?

It seems that the long-term experience with benefits of GFCF diets has contributed to general consensus that gluten and/or casein may be harmful for several individuals with ASD. GFCF diet has grown in popularity; however the mechanism explaining how it works is not clear. Some authors suggest that individuals with ASD have NFH. To evaluate an association between cytokine production with common dietary proteins as a marker of NFH and GI symptoms in children with ASD Jyonouchi *et al.* [49] investigated ASD children with (N = 75) or without GI symptoms (N = 34), children with NFH (N = 15), and control subjects (N = 19). Diarrhea and constipation were the major GI symptoms. They measured production of type 1 T-helper cells (Th1), type 2 T-helper cells (Th2), and regulatory cytokines by PBMC stimulated with whole cow's milk protein, its major components (casein, β-lactoglobulin, and α-lactoalbumin), gliadin, and soy. PBMC obtained from ASD children with GI symptoms produced more TNF-α/ IL-12 than those obtained from control subjects with cow's milk protein, β-lactoglobulin, and α-lactoalbumin. They also produced more TNF-α with gliadin, which was more frequently observed in the group with loose stools. PBMC obtained from ASD children without GI symptoms produced more TNF-α/IL-12 with cow's milk protein than those from control subjects, but not with β-lactoglobulin, α-lactoalbumin, or gliadin. Cytokine production with casein and soy were unremarkable. Based on these results Jyonouchi with coworkers concluded that a high prevalence of elevated TNF-α/IL-12 production with cow's milk protein and its major components indicates a role of NFH in GI symptoms observed in children with ASD.

Coeliac Disease and ASD

The best known gluten sensitive disorder is coeliac disease (CD). This clinically diagnosable disease is characterized by malabsorption and typical small-bowel mucosal atrophy. CD is an immune-mediated enteropathy caused by a permanent sensitivity to gluten in genetically susceptible individuals. Based on a number of studies in Europe and the United States, the prevalence of CD in children between 2.5 and 15 years of age in the general population is 3 to 13 per 1000 children, or approximately 1:300 to 1:80 children. Numerous studies demonstrate that children with CD frequently have GI symptoms such as diarrhea with failure to thrive, abdominal pain, vomiting, constipation and abdominal distension [60]. Currently the only available treatment is lifelong adherence to a GF diet.

Assessment for CD should be performed for any child with an ASD and GI symptoms. Testing at a minimum should include a total IgA level and tissue transglutaminase IgA antibodies [17]. The question thus arises: Have individuals with ASD also had CD? Some authors in the past reported the existence of a linkage of CD with ASD. Pavone *et al.* [61] evaluated 120 patients with CD diagnosed at the Pediatric Clinic of the University at Catania, Italy, in order to identify behavioral problems and autistic features: there were 20 controls for this part of the study. At the same time, CD was assayed in 11 patients with infantile autism and 11 age- and sex-matched controls. No CD case was detected among the group of autistic patients and subsequent antibodies determinations and jejunal biopsies gave normal results. Moreover none of the coeliac patients had a positive DSM-III-R test for infantile autism.

More recent study was performed at the Bologne University in 2008. They examined 150 patients with ASD and found CD in six of them. The authors thus recommend performing assessment for CD for any patient with ASD.

Recently, a significant association between maternal history of CD and ASD was observed in a cohort of 3 325 children with ASD in Denmark [62].

However, the experience with GF diet in patients with ASD show that in many cases the diet has beneficial effects and the symptoms of gluten sensitivity do not appear when gluten is reintroduced after several months of diet. Buie *et al.* [17] recommends that children on GF diet should consider testing for CD when gluten is reintroduced.

While CD is the hereditary life-long disease, there is a hope that symptoms of gluten sensitivity in autistic individuals might be treated. How is this possible? The experience show that gluten acts via different mechanisms in CD and ASD. CD is caused by an abnormal immune reaction to partially digested gliadin, one of the proteins from gluten. In CD gluten induces the secretion of autoantibodies which are targeted against transglutaminase 2. These autoantibodies are produced in the small-intestinal mucosa, where they can be found deposited extracellularly below the epithelial basement membrane and around mucosal blood vessels. In addition, during gluten consumption these autoantibodies can also be detected in patients' serum; disappears from the circulation on a GF diet, but remains for a long time in the small intestinal mucosal deposits [63].

While there are very detailed studies on chemistry and immunology of gliadin protein in CD, nothing is known about the participation of various epitopes of gliadin molecule in the autistic gut. Likewise, nothing is known about the defects of gluten and/or gliadin digestion in autistic intestine. Gliadin is extremely rich on glutamine; the sequences -Pro-Ser-Gln-Gln- and -Gln-Gln-Gln-Pro- were demonstrated to be common for toxic gliadin peptides [64,65]. Nothing is known about intestinal glutamine-glutamate homeostasis and the different functional roles of these closely related amino acids in intestinal mucosa in ASD.

Moreover, biologically active peptides derived from gliadin are tyrosine-containing peptides. The tyrosine-containing groups have the capacity to initiate damaging immunological reactions in patients with CD [66]. Tyrosine is the precursor for melatonin synthesis. The healthy intestine contains hundred times more melatonin than the brain. The synthesis of melatonin is compromised in individuals with ASD. These hypothetical lines only illustrate the complexity of possible interactions of gluten and gliadins in the GI system. These areas warrant further studies.

Opioid-Excess Theory

Another area of interest in connection with GFCF diet is the opioid-excess theory of ASD.

This theory suggests that some, but not all, of the symptoms ASD may be the consequence of the action of peptides of exogenous origin affecting neurotransmission within CNS. Gluten and casein are not broken down properly during digestion and small peptides with opioid activity are released from leaky gut to the blood, cross the BBB and enter the brain to exert an effect on neurotransmission, as well as producing other physiologically-based symptoms. There is a surprisingly long history of research accompanying this theory.

In 1979 Panksepp described a neurochemical theory of autism proposing that incomplete breakdown and excessive absorption of dietary food peptides may exert central opioid-like effects [15]. Panksepp thus put forward the idea that autism is an emotional disturbance arising from an upset in the opiate systems in the brain. This theory was extended by Shattock Sunderland group. Shattock and coworkers [67,68] suggest that the food-derived gut peptides may directly, or via formation of ligands, lead to disruption of normal neuroregulation and brain development. They tested many years whether the elimination of the proteins gluten and casein would improve behavior of children with ASD. They have established The Sunderland Protocol “that seeks to encourage the introduction and utilization of these interventions in a rational and logical way so as to maximize the benefits and minimize the chance of side effects or unnecessarily restrictive diets” [15].

The opiod-excess theory became popular among parents of children with ASD since it offers a seemingly simple explanation for understanding the cause of abnormal behavior of their children. It suggests that gluten and casein are harmful because they are sources of morphine-like compounds. The digestion of gliadin, protein of gluten, leads to peptides called gliadinomorphins. Proteins in bovine milk are also a common source of bioactive peptides. In the opioid-excess theory β-casomorphin from casein has the key role.

The biochemistry of casomorphins has been studied in details by Kaminski *et al.* [69]. He found that *in vitro* the bioactive peptide β-casomorphin 7 is yielded by the successive GI proteolytic digestion of bovine β-casein variants A1 and B, but this was not seen in variant A2. In hydrolysed milk with variant A1 of β-casein, β-casomorphin 7 level is four-fold higher than in A2 milk. Variant A1 is the most frequent in milk of Red, Ayrshire, and Holstein-Friesian cattle breeds. In contrast, a high frequency of A2 is observed in milk of Guernsey and Jersey cattle. Epidemiological evidence from New Zealand claims that consumption of β-casein A1 is associated with higher national mortality rates from ischaemic heart disease. These interesting findings demonstrate that differences in casein polymorphism might also play important, but unknown variable, in evaluation of the requirement of CF diet.

To a significant degree, this bioactive peptide crosses GI mucosa and enters blood in certain individuals. These compounds enter the circulation, cross the BBB, and influence neurological functioning [70]. The level of β-casomorphin 7 is elevated significantly in urine and blood of patients with schizophrenia and autism [71,72].

The presence of opioid peptides in urine has been investigated as support for the opioid-excess theory. Reichelt and Knivsberg [73] reported the findings of opioid peptides derived from food proteins in urine of ASD patients. They suggested that this may be due to a genetically based peptidase deficiency, which manifests by a dietary overload of exorphin precursors, such as by increased gut uptake. (The enzyme dipeptidyl peptidase is required for break down casomorphins into inactive dipeptides.) These authors also show highly significant decreases in urine peptides after introducing a GFCF diet in children who were followed for 1- 4 years [74].

The urine analyses have been extensively provided by Sunderland group. They repeatedly observed the elevated levels of trans-indolyl-3-acryloylglycine (IAG) in the urine of people with autism and Asperger syndrome and reported that their results strongly suggest that urinary titers of IAG may constitute an objective diagnostic indicator for ASD [15, 75-77].

In contrast, some researchers failed to confirm the presence of metabolites of gluten or casein in urine. Dettmer *et al.* [78] developed method to analyze gliadinomorphin and β-casomorphin in urine. The method was used to screen 69 urine samples from children with and without ASD for the occurrence of neuropeptides. The target neuropeptides were not detected above the detection limit in either sample set. Cass *et al.* [79] found no significant differences between the HPLC urinary profiles of 65 boys affected by autism and 158 typically developing controls. In those cases where HPLC showed peaks in the locations at which opioid peptides might be expected to be found, they established that these peaks did not, in fact, represent opioid peptides. These authors concluded that given the lack of evidence for any opioid peptiduria in children with autism, opioid peptides can neither serve as a biomedical marker for autism nor be employed to predict or monitor response to a CF and GF diets. The significance of reports of increased levels of metabolites of casein and gluten in the urine of people with ASD remains unclear [80]. Urinary peptides are not used in conventional practice to prescribe or monitor dietary restriction.

MET: A POSSIBLE GENETIC CAUSE FOR THE ASSOCIATION OF ASD AND GI DISEASE

It seems that genetic research offers the important information, which could contribute to endless discussions whether the association of ASD and GI disorders does exist. In 2009, Campbell *et al.* reported that a functional variant in the promoter of the *MET* gene encoding the MET (mesenchymal epithelial transition factor) receptor tyrosine kinase (RTK) located within a chromosome 7q31 autism candidate gene region, is associated with ASD and that disrupted MET signaling may contribute to the risk for ASD that includes familial GI dysfunction [81]. The MET RTK participates not only in development of the cerebral cortex and cerebellum, both of which may be altered in ASD (see Chapter 2), but also contributes to GI repair and immune function, disruptions of which co-occur in some patients with ASD.

Autism Susceptibility Locus on Chromosome 7q and MET Gene

The International Molecular Genetic Study of Autism Consortium (IMGSAC) found evidence for an autism susceptibility locus (AUTS1) on chromosome 7q. The IMGSAC was the first to publish a genome-wide linkage screen for autism, and the first to identify a linkage peak on the chromosome 7q region [82]. Campbell [23] explains a number of reasons for the difficulty in identifying the chromosome 7q autism risk genes, including genetic and

THE SEARCH FOR THE ROLE OF OT IN THE ETIOLOGY, PATHOGENESIS, AND THERAPY OF ASD

A number of researchers have suggested that OT might be implicated in the etiology of ASD, which is characterized by severe social impairment [17, 22-34]. Baron-Cohen *et al.* [18] showed amygdala activation in response to the RMET using fMRI. Some areas of the prefrontal cortex also showed activation when using social intelligence. In contrast, patients with autism activated the fronto-temporal regions but not the amygdala when making mentalistic inferences from the eyes. Hollander noticed that 60 % of the autistic patients had OT treatment during labor and postulated hypothesis that excess OT, possibly through OT administration at birth, could contribute to the development of ASD [32,33]. Interestingly, Hollander and his colleagues are the first to have used both intravenous and intranasal delivery to study the behavioral effects of OT in ASD. Infusions of synthetic OT and Pitocin significantly reduced repetitive behaviors in adult patients with autistic and Asperger syndrome. The questions thus have risen: Does OT evoke or ameliorate ASD? Do autistic children have too much or too little OT in their brains?

Increased OT during Birth

The first speculations regarding the contribution of OT in ASD appeared in connection with the use of OT at birth. The knowledge of the classical physiological role of OT to stimulate uterine smooth muscle contraction at parturition led to the widely spread administration of OT to induce labor or to spur labor that's going slowly. OT is administered under the trade names such as Pitocin or Syntocinin for labor induction and/or labor augmentation during childbirth. Hattori *et al.* [22] reported that children born in one Japanese hospital routinely using a combination of anesthetics and synthetic OT - nonapeptid Pitocin - had significantly higher rate of autism than children born in three other hospitals, which rarely used general anesthesia. Laila Y. Al-Ayadhi [35] also found a higher incidence of Pitocin-induced labor among autistics children from Riyadh – Saudi Arabia as compared to normal. No indication for a relationship between labor induction and autism can be found in Fein's study [36]. These researchers examined 478 preschool children with autism (51 high functioning non-verbal IQ>79, 123 low functioning), language disorders (197) and low-IQ (107). Amongst the high functioning autistic children, 21.6 % were found OT-induced, amongst the low functioning autistic 24 %, amongst the language disordered 19.3 % and amongst the low IQ 27.1 % were found induced [37]. When compared to the U. S. national average of labor induction, which has been used in 20 % of mothers, no indication for a relationship between labor induction and autism can be found.

No correlation between Pitocin at labor and prevalence of ASD was found by Gale *et al.* [38]. These researchers examined the rates of labor induction using Pitocin in 41 children with autism and age-matched controls (15 typically developing and ten with mental retardation). There were no differences in Pitocin induction rates as a function of either diagnostic group (autism versus control) or IQ level (average versus sub average range), failing to support an association between exogenous exposure to OT and neurodevelopmental abnormalities.

Neurologists Woodward, in a personal letter to ARI notes that it seems improbable that a single dose of OT, an endogenous hormone naturally present during labor, would lead to cerebral maldevelopment [39]. Two barriers are commonly thought of as preventing OT from potentially accessing the infant's brain: The maternal placenta barrier and the blood-brain barrier (BBB) of the infant. However, it should be considered that during labor, a unique stress situation exists for both mother and child, which could result in the release of cytokines and create an oxidative stress situation which has been shown to render the BBB more permeable than usual. Also, the BBB in the infant might not be as fully developed as compared to adults and might therefore be more permeable towards small lipid insoluble molecules. Wahl [37] offered the hypothesis in support of Hollander's hypothesis that excess OT, possibly through OT administration at birth, could contribute to the development of ASD by proposed down regulation of the OTR. He proposed that to test this hypothesis, the OT concentrations in the fetal blood and brain should be routinely monitored right after birth. Furthermore, at a molecular level in a large clinical test study, OT could be administered in a radiolabeled form, preferably [^{3}H]-, but also [^{125}I]- or [^{131}I]-OT, and its occurrence/non-occurrence in the infant's brain should be determined by radiotracing. Therefore, the issue appears to remain open for further research and debate.

The evidence that OT not only has a critical role in birth and lactation but also in the emergence of an intimate bond with offspring is related to OT's reported effects on animal behavior, favoring social bondage, notably in sheep, voles, rats, and especially mice.

Although research on the neurobiological foundation of social affiliation has implicated the neuropeptide OT in processes of maternal bonding in mammals, there is little evidence to support such links in humans. Feldman *et al.* [40] sampled plasma OT and cortisol of 62 pregnant women during the first trimester, last trimester, and first postpartum month. OT was assayed using enzyme immunoassay, and free cortisol was calculated. After the infants were born, their interactions with their mothers were observed, and the mothers were interviewed regarding their infant-related thoughts and behaviors. OT was stable across time, and OT levels at early pregnancy and the postpartum period were related to a clearly defined set of maternal bonding behaviors, including gaze, vocalizations, positive affect, and affectionate touch; to attachment-related thoughts; and to frequent checking of the infant. This study demonstrated that OT level may play a role in the emergence of behaviors and mental representations typical of bonding in the human mother across pregnancy and the postpartum period. Experimental research showing that OT improves 'mind reading' suggests that OT may facilitate parental sensitivity at any stage in parents' lives and not only during the period around birth [20].

Circumstantial evidence for the potentially important role of OT in human parenting may be derived from experimental studies administering OT to patients with autism, which enhanced their social cognitions and empathic feelings [41]. Further studies relating autism to variations in the OTR gene will also document that the hypothesis of Pitocin administration during labor as the cause of ASD cannot give a satisfying explanation for direct involvement of OT in the etiology of ASD.

OT has been coined as the hormone of love and cuddle. Can we suggest that the mothers with low endogenous production of OT need primarily exogenous OT to support labor? Such simplification could be seemingly in concordance with the oldest Kanner´s hypothesis. Leo Kanner was calling attention to what he saw as a lack of parental warmth and attachment to their autistic children. In his 1949 paper, he attributed autism to a "genuine lack of maternal warmth" and the "Refrigerator Mother" theory of autism was born [42].

Jane Strathearn and colleagues [43] examined 30 first-time new mothers to test whether differences in attachment, using a modified version of the Adult Attachment Interview, a semistructured interview that assesses a person's childhood interactions and bonding experience with their primary caregivers (generally parents). On viewing their own infant's smiling and crying faces during fMRI scanning, mothers with secure attachment showed greater activation of brain reward regions, including the ventral striatum, and the OT-associated hypothalamus/pituitary region. Peripheral OT response to infant contact at 7 months was also significantly higher in secure mothers, and was positively correlated with brain activation in both regions. Insecure/dismissing mothers showed greater insular activation in response to their own infant's sad faces. These results show that individual differences in maternal attachment may be linked with development of the oxytocinergic neuroendocrine systems.

Decreased Plasma Levels of OT in Autistic Children

Although making inferences to central OT functioning from peripheral measurement is difficult, the data suggest that OT abnormalities may exist in autism. Modahl *et al.* [29] found significantly lower levels of plasma OT in 29 children diagnosed with autism compared with 30 age-matched healthy control children. OT increased with age in the normal but not the autistic children. Elevated OT was associated with higher scores on social and developmental measures for the normal children, but was associated with lower scores for the autistic children. A follow-up study of these same subjects revealed that the differences in plasma OT levels were associated with an increase in incompletely processed OT fragments, suggesting that peptide processing may be dysregulated in the autistic patients [31]. The hypothalamic synthetic pathway of nonapeptide OT involves the synthesis of carboxy-extended forms that serve as intermediate prohormones. A prohormone is sequentially processed to peptides. These peptides are the bioactive amidated form and the carboxy- extended peptides, OT-Gly, OT-Gly-Lys and OT-Gly-Lys-Arg. Using OT antisera with different specificity for the peptide forms, Green *et al.* measured plasma OT and carboxy-extended peptides in each group of subjects. T tests showed that there was a decrease in plasma OT and an increase in extended peptides compared with control subjects. Green with co-workers [31] concluded that observed deficits

in OT peptide processing in children with autism may be important in the development of this syndrome. However, it is not known whether the carboxy-extended OT prohormones have some biological activity and could compete with OT for receptor binding in human brain. In peripheral tissues they only serve as a substrate for OT synthesis and do not compete with OT for OTR binding [44].

The second study found lower level of OT in autistic children in Central Saudi Arabia [35]. Seventy-seven autistic children with an age ranging from 3.5-14 years from Riyadh area participated in the study, with the confirmed diagnosis according to DSM-IV diagnostic criteria of autism. Results showed a statistically significant lower plasma level of OT in autistic group (0.074 ± 0.01 ng/ml), as compared to the control group (0.107 ± 0.01 ng/ml). Further more, vasopressin plasma level was significantly lower in autistic children. There was no significant correlation between the degree of autism, or the age of the affected child and plasma levels of OT or vasopressin.

Marchini and Stock [45] investigated OT profile in 26 healthy newborn (1-day-old) infants using the pulse detection program PULSAR. The plasma OT concentrations were determined by specific radioimmunoassay. They found that 42 % of the infants presented one peak in the OT level during a 4-min period. The peak constituted a 111 ± 66 % increment of the baseline value. These researchers suggested that the release of OT during basal conditions occurs in a pulsatile way in newborn infants and that these hormone pulses reflect fluctuations in the activity of the hypothalamic neurosecretory cells.

However, measurements of OT concentration in blood using a sensitive enzyme immunoassay (EIA) are infrequently performed. Carter *et al.* [46] described the method for measurements of biologically relevant changes in salivary OT. Their results confirm the biological relevance of changes in salivary OT with stressors and support saliva as a noninvasive source to monitor central neuroendocrine function. In the near future it may become easier to measure salivary OT as a biomarker for affiliative behavior in humans, which would enable direct tests of the association between OT and ASD.

The Effects of OT Administration in Adult Autistic Patients

Several animal studies, mostly with rodents, investigated the effect of OT administered centrally or peripherally, and found that OT facilitated social recognition [47]. Fergusson *et al.* [48-50] observed that male mice with a null mutation in the gene coding for OT ("OT knockout mice") failed to recognize a conspecific even over repeated exposures and that a single intracerebroventricular injection of OT before the initial encounter with the conspecific enabled social memory acquisition. No studies have yet examined the pharmacological influences of OT on the social deficits in autism; however, infusions of synthetic OT and Pitocin significantly reduced repetitive behaviors in adult patients with autistic and Asperger syndrome. Hollander *et al.* [32] investigated the influence of OT infusion on repetitive behaviors in autism. In a double-blind cross-over design, 15 ASD adult patients showed a significant reduction in repetitive behaviors — need to know, repeating, ordering, need to tell/ask, self-injury, and touching — after OT infusion versus placebo infusion.

Inspired by these findings, Hollander with coworkers [33] investigated further whether increased levels of OT would facilitate social information processing for adult individuals diagnosed with autism or ASD. They focused on auditory processing of social stimuli—and specifically participants' ability to assign affective significance to speech—because this deficit is present in most autistic people, and it has been hypothesized that its disruption could be central to the social and speech deficits in autism. In this randomized, placebo-controlled, double-blind crossover investigation, 15 adults (14 men; mean age 32.9 years, range 19–56 years) diagnosed with autism (n = 6) or Asperger syndrome (n = 9) received OT and placebo challenges during visits separated by a minimum of one week. Participants were medically healthy and medication-free for at least two weeks before and throughout the study. Each subject served as his or her own control and completed both OT and placebo challenges on separate days; synthetic OT (Pitocin) or placebo was continuously infused over a 4-hour period. Eight subjects received OT first, and seven received placebo first. Comprehension of affective speech was tested at baseline, just before the intravenous OT/placebo infusion, and at 30, 60, 120, 180, and 240 min over the course of the infusion. In this study, participants were presented with four sentences of neutral content: "The boy went to the store," "The game ended at 4 o'clock," "Fish can jump out of the water," and "He tossed the bread to the pigeons." The sentences were pre-recorded and played for participants on a tape player; the voice reading the sentences was unknown to participants

and was the same for all participants. Each sentence was presented with one of four emotional intonations (happy, indifferent, angry, and sad) with the pairing of emotional expression and sentences in one of six counterbalanced orders. Participants were then asked to indicate the emotional mood of the speaker by pointing to the word that corresponded to the emotion that they believed matched the one they heard on the tape; the examiner recorded participants' responses for all trials. As it turned out, this task was relatively easy for the adult participants in this study and, consequently, the findings were negatively skewed. In an effort to reduce the negative skew of the variable and to better balance the difficulty of the task, the outcome measure was scored dichotomously as 1 (all items correct) and 0 (not all items correct) [33]. This study found that OT administration facilitated the processing and retention of social information in adults diagnosed with autism or Asperger syndrome: compared with subjects who received placebo first, subjects who received OT first showed increased retention of affective speech comprehension after a delay. These authors pointed that it would be interesting to explore the effects of OT on other aspects of social cognition, to investigate whether different methods of administration (i.e., intranasal versus intravenous) yield different results and to investigate whether cerebrospinal fluid OT levels differ depending on the method of administration used.

Treatment studies with standardized measures to assess "real-life" improvements in social functioning are also needed to demonstrate the practical utility of OT in the treatment of autism. Finally, studies are needed to investigate the effects of OT administration in younger children who could potentially benefit from early intervention.

The more recent studies have brought demonstration that intranasal OT improves emotion recognition for youth with ASD [51]. In a double-blind, randomized, placebo-controlled, crossover design, OT nasal spray (18 or 24 IU) or a placebo was administered to 16 male youth aged 12 to 19 who were diagnosed with autistic or Asperger syndrome. Participants then completed the RMET, a widely used and reliable test of emotion recognition. These researchers found that OT intranasal administration improved performance on the RMET. This effect was also shown when analysis was restricted to the younger participants aged 12 to 15 who received the lower dose. These findings suggest the potential of earlier intervention and further evaluation of OT nasal spray as a treatment to improve social communication and interaction in young people with ASD.

Recently, Andari *et al.* [52] investigated the behavioral effects of OT in 13 individuals with high-functioning autism or with Asperger syndrome in a simulated ball game where participants interacted with fictitious partners. These researchers found that after OT inhalation, patients exhibited stronger interactions with the most socially cooperative partner and reported enhanced feelings of trust and preference. Andari with colleagues thus support a therapeutic potential of OT through its action on a core dimension of autism.

INVESTIGATIONS OF OTR IN ASD

It is interesting to note that OT genes belong to the oldest ones; they should be even older than 500-700 million years [3,53]. Despite the great diversity of the proposed functions of OT and the oxytocinergic system, only one type of OTR has been identified both in laboratory animals as well as in humans [54-56].

Biochemistry of OTR

The encoded OTR is a 388-amino-acid polypeptide, which belongs to the family of G protein-coupled receptors (GPCR) [57]. All GPCR display seven heptihelical domains that are hydrophobic and span lipid bilayer (see Fig. **3**). Extracellular domains and core of bundle of seven transmembrane segments act in signal discrimination and ligand binding [58,59]. The affinity of the receptor for ligands is strongly dependent on the presence of divalent cations and cholesterol that both act like positive allosteric modulators. Notably, some evidence is provided that OTR are also present in the form of dimeric or oligomeric complexes at the cell surface.

Intracellular domains of OTR function in signal propagation to heterotrimeric G proteins. Heterotrimeric G proteins are constructed of three types of subunits, an α-subunit uniquely capable of binding and degrading GTP and a tightly knit complex of β- and γ-subunits. The nomenclature now popularly known as Gq, Gs, and Gi classes determines the interaction of the α-subunit with various effectors molecules [60]. Gs means that the α-subunit of heterotrimeric G protein interacts with adenylyl cyclase (AC) and stimulates the production of cyclic adenosine

monophosphate (cAMP), while Gi inhibits the production of cAMP. Gq has been used for a class of G proteins, which activate phospholipase C (PLC). OTR is able to couple to different G proteins with a subsequent stimulation of various signaling cascades [61-64] (see Fig. **3**). In spite of the fact that there is only one type of OTR, its stimulation can produce an array of cell functions, and moreover, sometimes in an opposite manner. Because of dependence on G protein coupling, OTR can give rise to opposite effects on the same cellular function. For example, OTR coupling to Gq induces the contraction in myometrial cells, while OTR activation of Gi decreases preterm labor [65].

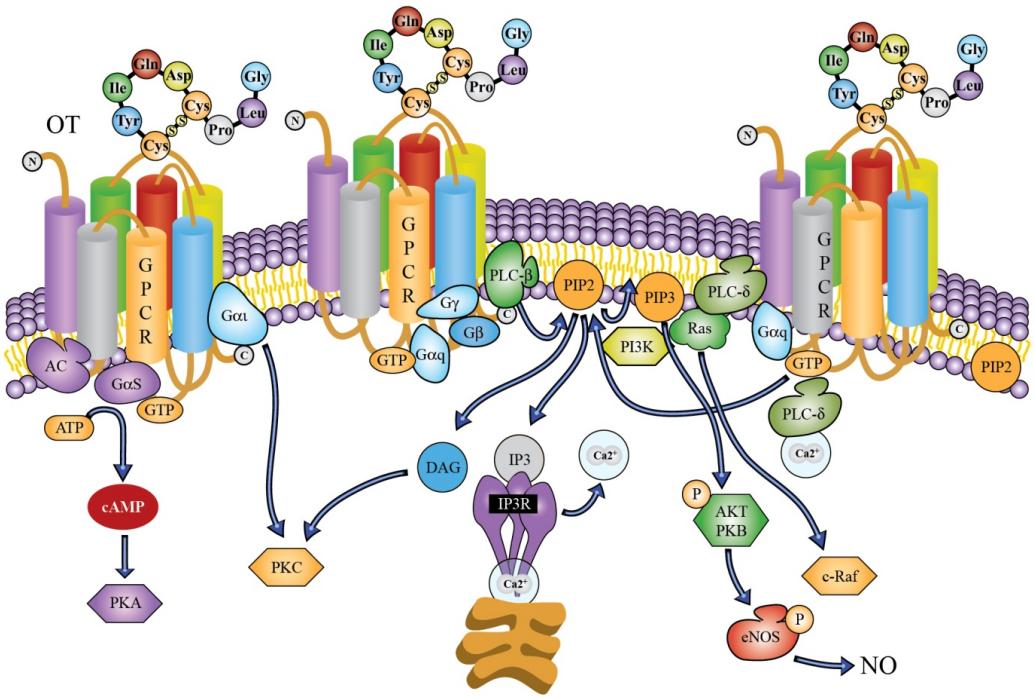

Figure 3: Various signaling pathways used by OTR.

Interesting findings concerning the coupling of OTR with various G proteins were reported in the brain. To investigate the potential role of Gq signaling in behavior, Wettschureck *et al.* [66] generated mice, which lack the α-subunits of the two main members of the Gq/11 family, selectively in the forebrain. These authors found that forebrain Gq/11-deficient females did not display any maternal behavior such as nest building, pup retrieving, crouching, or nursing. However, olfaction, motor behavior, and mammary gland function were normal in forebrain Gq/11-deficient females. It seems therefore that Gq/11 signaling is indispensable to the neuronal circuit that connects the perception of pup-related stimuli to the initiation of maternal behavior.

Several findings indicate that the OTR is regulated in a very complex manner. For example, it has been shown that OTR in plasma membrane mediates the inhibition of cell proliferation while OTR localized in caveolin-enriched microdomains (lipid rafts) mediates a mitogenic effect [63,67]. The search of new OT analogs could bring a class of highly selective compounds with therapeutic relevance in obstetrics, oncology, psychiatry, and some other areas of medicine. On the other hand, it is difficult to predict the effects of exogenous administration of OT or its synthetic analogues.

Genetic Variants in the OTR Gene Associated with ASD

Animal models and linkage data from genome screens indicate that the *OTR* gene is an excellent candidate for research concerning psychiatric disorders, particularly those involving social impairments, such as autism. Some modest evidence suggesting a possible association of the *OTR* gene with autism has been already presented.