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# Biomarkers-Directed Strategies to Treat Autism

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Afaf El-Ansary and Hussain Al Dera

Additional information is available at the end of the chapter

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## Abstract

Autism is a neurodevelopmental disorder characterized by social, communication, and behavioral symptoms. Recent research has attempted to identify the potential mechanisms that may contribute to the pathogenesis of autism. Biomarkers as noninvasive quantitative biological measures with accurate indication of a specific mechanism can lead to a better understanding of the pathogenesis required to design the most effective treatments of autism. There is also great hope that the discovery of valid and predictive biomarkers for this disorder will help earlier and more targeted methods for diagnosis and intervention. In this chapter, we discuss some of the current theorized mechanisms contributing to autism, including inflammation, oxidative stress, impaired detoxification, glutamate excitotoxicity, gut-microbiota-brain axis, impaired fatty acid profiling, and serotonin (5-HT)/oxytocin (OT) abnormalities as target to treat autism. Moreover, based on our understanding of the role of these mechanisms, selected treatment strategies are suggested. These strategies include nutraceuticals, probiotics/prebiotics and  $\omega$ -3 supplementation, targeting glutamate transporters or selective 5-HT reuptake inhibitors, and intranasal OT treatment. Of course, the joint efforts of scientists, caregivers, and other stakeholders must combine to identify valid, clinically useful autism biomarkers that may lead to efficient treatment strategy and/or combined strategies.

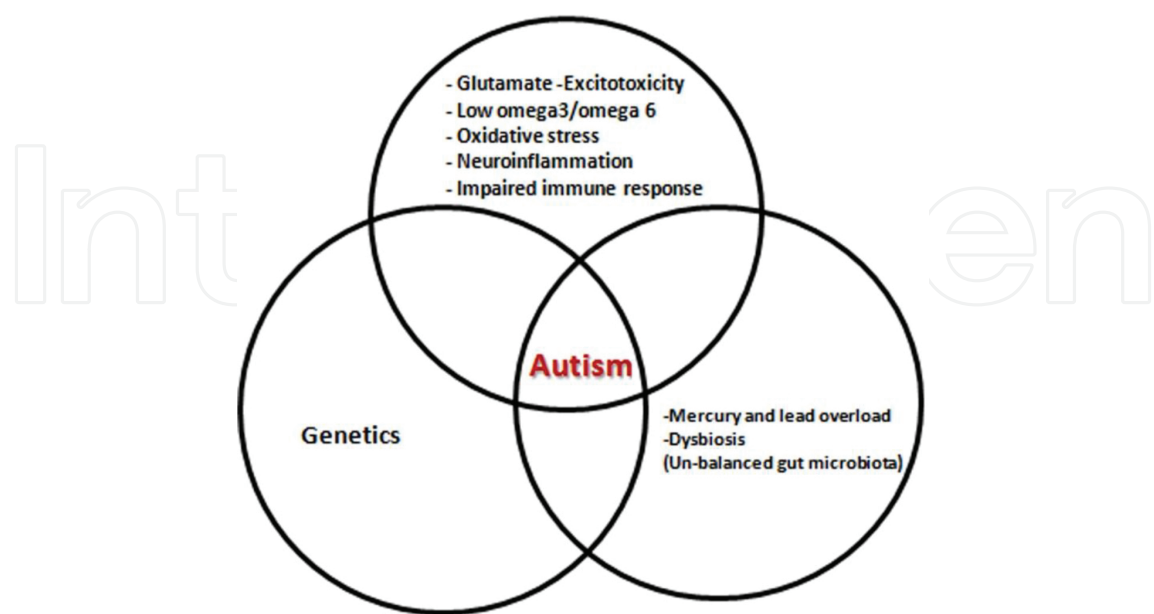
**Keywords:** autism, biomarkers, excitotoxicity, neuroinflammation, nutraceuticals,  $\omega$ -3, oxidative stress, probiotic

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## 1. Introduction

Autism is a neurodevelopmental disorder characterized by deficits in cognition and learning, behavior, social interaction, and communication. Among the challenging behaviors that negatively affect the child with autism is the social interaction impairment. Social interaction is defined as how an individual uses verbal and nonverbal communication during interperso-

nal exchanges. Children with autism usually present difficulty in recognizing the thoughts and feelings of others, and this is described as “mind blindness,” which can lead to ineffective communication [1]. Cognitive differences are also prevalent in children with autism. As a result, academic differences often exist due in part to weaknesses with executive functioning. The current management strategies, practice, and methods are put into place by professionals, researchers, and parents to compensate for the deficits in these areas. They are designed and selected in hopes of producing the maximum improvement in autistic behavior. Genetic heterogeneity was recorded earlier in twin, family, and linkage studies. The autism-related genes seem to be contributed on few etiological pathways related to detoxification, synaptic function, and neurogenesis [2–5]. As genes and environment rarely act independently to induce autism, the interaction between both at various developmental times usually plays an important role in the pathology of this disorder. Because development is a dynamic process, a constant interplay between genes and environment usually occurs [6]. Although the progressive increase in the prevalence of autism can be attributed to the increase in public awareness and the broadening of diagnostic construct, of course, it can be also related to the incidence of environmental factors [7, 8] and their interactions with yet unknown genetic vulnerability. Nutrients, heavy metal pollution, medications, and pesticides as the most commonly examined exposures during pregnancy are among the environmental neurotoxic insult on developing brains. Understanding how low-level chemical exposures influence the molecular, cellular, and behavioral outcomes relevant to autism will provide insight regarding gene-environment interactions and possibly yield novel intervention strategies. There is no metabolic biomarker or panel of markers that can precisely define autism, but examining different signaling pathways to identify any abnormalities in autistic patients compared to their normal peers can be enhanced to treat autism through the amelioration of these defected pathways.



**Figure 1.** Role of genetic, environmental, and biological mechanisms in the etiology of autism.

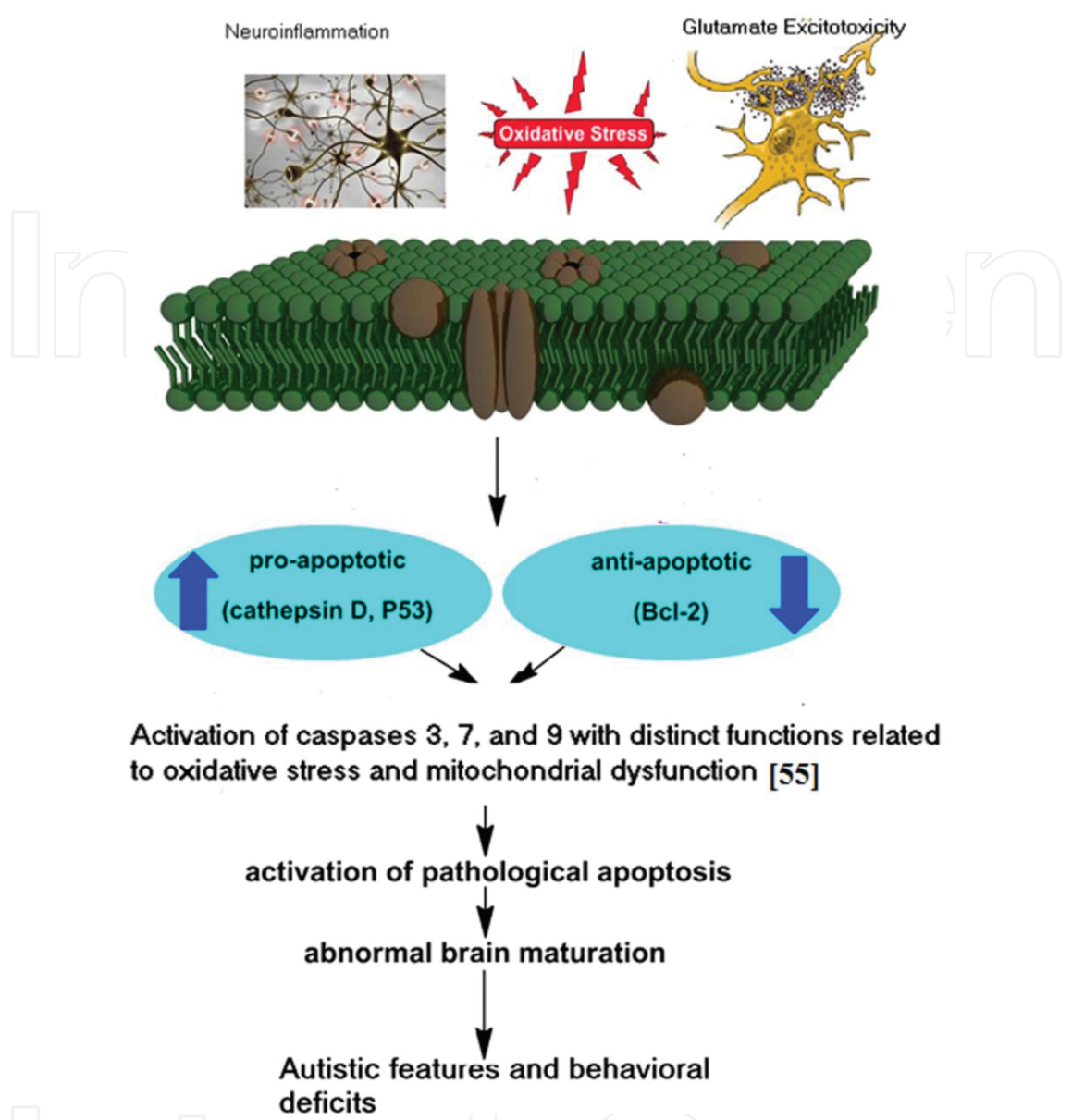
Many studies related to the screening of biomarkers of autism were successful enough and reproducible to ascertain the role of oxidative stress, environmental toxicants, mitochondrial dysfunction, and immunology/inflammation as four main etiopathological mechanisms of autism. In addition, there is accumulating evidence pointing to the contribution of lipid abnormalities, gut microbiota, and glutamate excitotoxicity in generating biomarkers related to autism. **Figure 1** demonstrates the relationship between genetic, environmental, and biological mechanisms in the etiology of autism.

## 2. Biomarkers related to autism

Screening for antioxidants includes the measurement of glutathione (GSH) as the primary antioxidant entrusted with the protection against oxidative stress, neuroinflammation, and mitochondrial damage. GSH is critically important in regulating the detoxification mechanism and modulating the production of oxidative stress-related parameters. Measuring reduced GSH, oxidized GSH (GSSG), or GSH status (GSH/GSSG) proves to be of great use in the determination of the patient's oxidation status. In a trial that evaluated the detoxification mechanism in autism, a systematic review of 39 studies was conducted. It proved that many patients with autism have lower GSH/GSSG, indicating poor antioxidant and detoxification mechanisms [9]. Among the recorded detoxification markers that are studied in autism are *p*-hydroxyphenyllactate, pyroglutamate, benzoate, and hippurate. Elevated levels of hippurate and benzoate are related to impaired phase II detoxification via glycine conjugation [10]. Abnormal levels of pyroglutamate can indicate an impairment of GSH metabolism and a depleted GSH status.

In more recent studies, serum thioredoxin levels and F<sub>2</sub>-isoprostane are related to cognitive and social impairment severity in autistic patients [measured by Childhood Autism Rating Scales (CARS) or Social Responsiveness Scale (SRS), respectively] [11–13]. A negative correlation between GSH peroxidase and CARS has also been reported [11]. Many studies prove that oxidative stress can easily be related to chronic inflammation, glutamate excitotoxicity, and increased mitochondrial dysfunction as etiological mechanisms in autism [14]. Additionally, the observed cellular damage in these patients may range from structural damage and mitotic arrest to apoptosis and cell necrosis depending on the severity of oxidative stress.

**Figure 2** demonstrates the suggested relationship between these etiological mechanisms and apoptosis of neurons that might lead to abnormal brain maturation that presents as autistic features and behavioral deficits. Glutamate excitotoxicity, oxidative stress, and neuroinflammation are signaling pathways that might cause an increase and/or decrease of proapoptotic and antiapoptotic proteins, respectively. Under the effect of genetic change or the environment (e.g., heavy metal toxicity and altered gut microbiota), the activation of pathological apoptosis (elevation of caspases) impairs normal brain maturation and induces autistic phenotype.

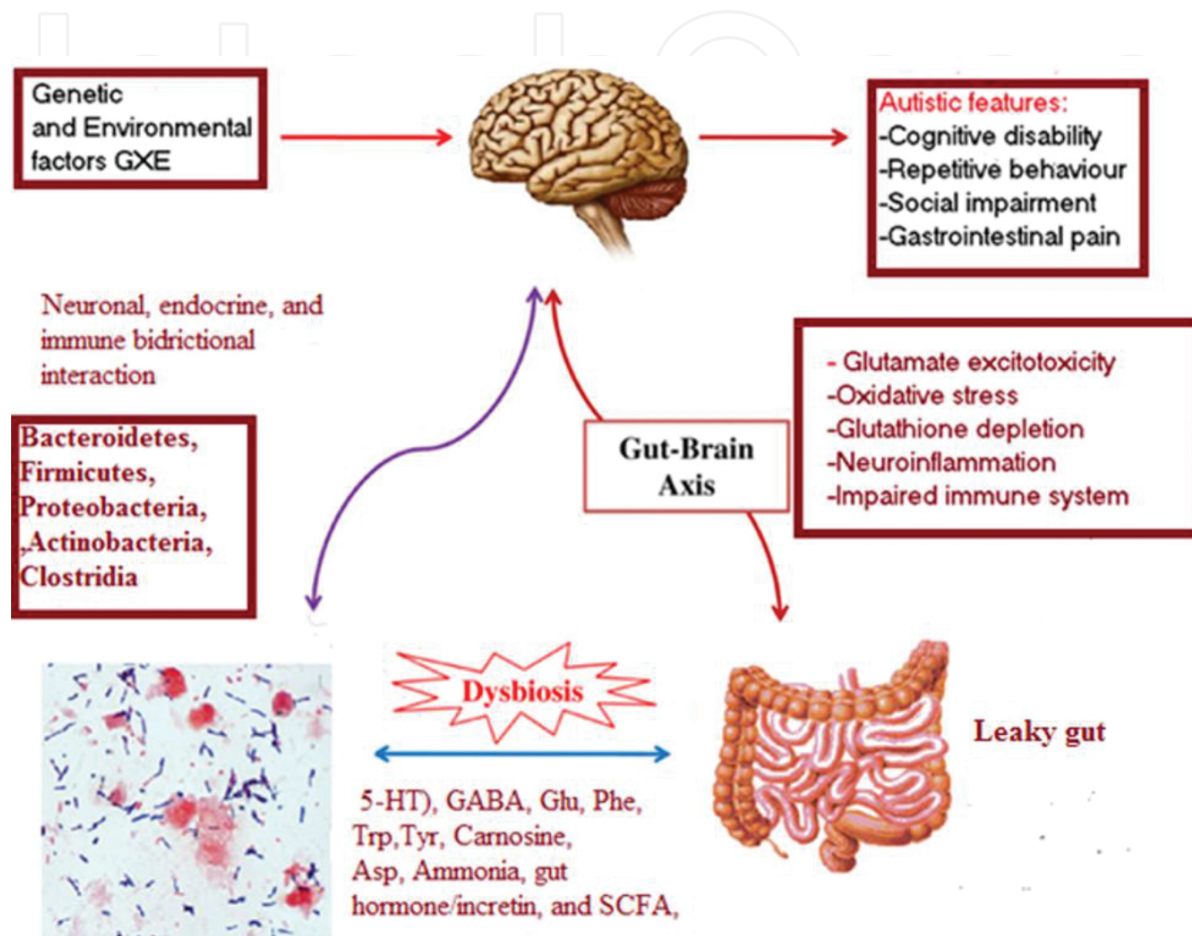


**Figure 2.** Suggested relationship between oxidative stress, glutamate excitotoxicity, neuroinflammation, and pathological apoptosis leading to autistic phenotype.

The realization that the microbiota-gut-brain axis plays a critical role in the etiology of autism has recently emerged. The regulation of this axis is essential for maintaining homeostasis, including that of the central nervous system (CNS). Nowadays, understanding microbiota-brain interactions has become exciting area of research, which may contribute new insights into individual variations in cognition, social interaction, mood, and sleep disorders as characteristic features of patients with autism [15, 16]. The ability of gut microbiota to communicate with the brain and in turn induce behavioral changes is emerging as an interesting concept in autism. The brain-gut axis is a bidirectional communication system composed of neural pathways, such as the enteric nervous system (ENS), vagus, sympathetic, and spinal nerves, as well as humoral pathways, which include cytokines, hormones, and neuropeptides



as signaling molecules [16]. Brain excitotoxicity, oxidative stress, and neuroinflammation can directly or indirectly affect the composition of the gut microbiota. On the contrary, microbial overgrowth and their metabolites can modulate the brain normal function. **Figure 3** demonstrates the suggested bidirectional interaction between the gut-microbiota and the brain as an etiological mechanism in autism.



**Figure 3.** Gut-brain axis: pathways of communication between brain and gut microbiota.

This fact was confirmed by a study that has suggested a significant risk of autism in children born to mothers with severe infections during pregnancy [17]. Additionally, offspring of pregnant female monkeys exposed to antibodies produced postinfection usually develop pathologies of the CNS and exhibited behavioral changes similar to those seen in autistic children [18]. Autoantibodies triggered by systemic inflammation are generated during pregnancy and are now accepted to play a role in abnormal neurological and impaired blood-brain barrier (BBB) development in the fetus with a concomitant increased risk of autism [19]. The molecular trigger for autoantibody generation is poorly understood, but there is a possibility that autoantibodies against receptors for key microbiota metabolites, such as serotonin (5-HT),  $\gamma$ -aminobutyric acid (GABA), glutamate, tryptophan, and short-chain fatty acids (SCFA), may be directed by the immune system under conditions of aberrant metabolite

accumulation in the blood as well as inappropriate immune system in early life or mimicry by gut bacteria [20].

These early observations provide a potential explanation for the gastrointestinal problems suffered by patients with autism. In support of this line of thinking, children with autism are shown to have increased permeability of the gastrointestinal tract, called “leaky gut,” causing microbial products to escape into the bloodstream and possibly reach the brain [21]. These products alter the immune system, resulting in a progression of the disease [22]. Differences in the gut microbiota between maternal infection activated (MIA) offspring and controls are observed due primarily to changes in the diversity of Clostridia and Bacteroidia [23]. The intestines of some autistic patients with intestinal abnormalities are known to bear *Sutterella* and *Clostridium bolteae* [24], organisms lacking in control populations with similar gastrointestinal problems, together with lower *Bifidobacterium* and *Lactobacillus* species. **Table 1** demonstrates altered gut microbiota in individuals with autism in relation to social impairment.

Autism
Significantly ↑ in <i>Clostridium histolyticum</i> [24]
Significantly ↑ in Bacteroidetes [25]
Significantly ↓ in species of <i>Bifidobacterium</i> and ↑ in <i>Lactobacillus</i> [26]
Significantly ↓ in species of <i>Bifidobacterium</i> spp. [27]
Significantly ↓ in Bacteroidetes, ↑ in Firmicutes: Bacteroidetes, ↑ in Betaproteobacteria [28]
Significant ↑ in <i>Sutterella</i> spp. [29]
Significant ↑ in <i>Clostridium</i> [30]
↑ in <i>Clostridium</i> , Bacteroidetes, <i>Prophyromonas</i> , <i>Prevotella</i> , <i>Pseudomonas</i> , <i>Aeromonas</i> , and Enterobacteriaceae; ↓ in <i>Enterococcus</i> , <i>Lactobacillus</i> , <i>Streptococcus</i> , and <i>Staphylococcus</i> [31]

**Table 1.** Association between gut microbiota altered composition and social impairment as core symptom in autistic patients.

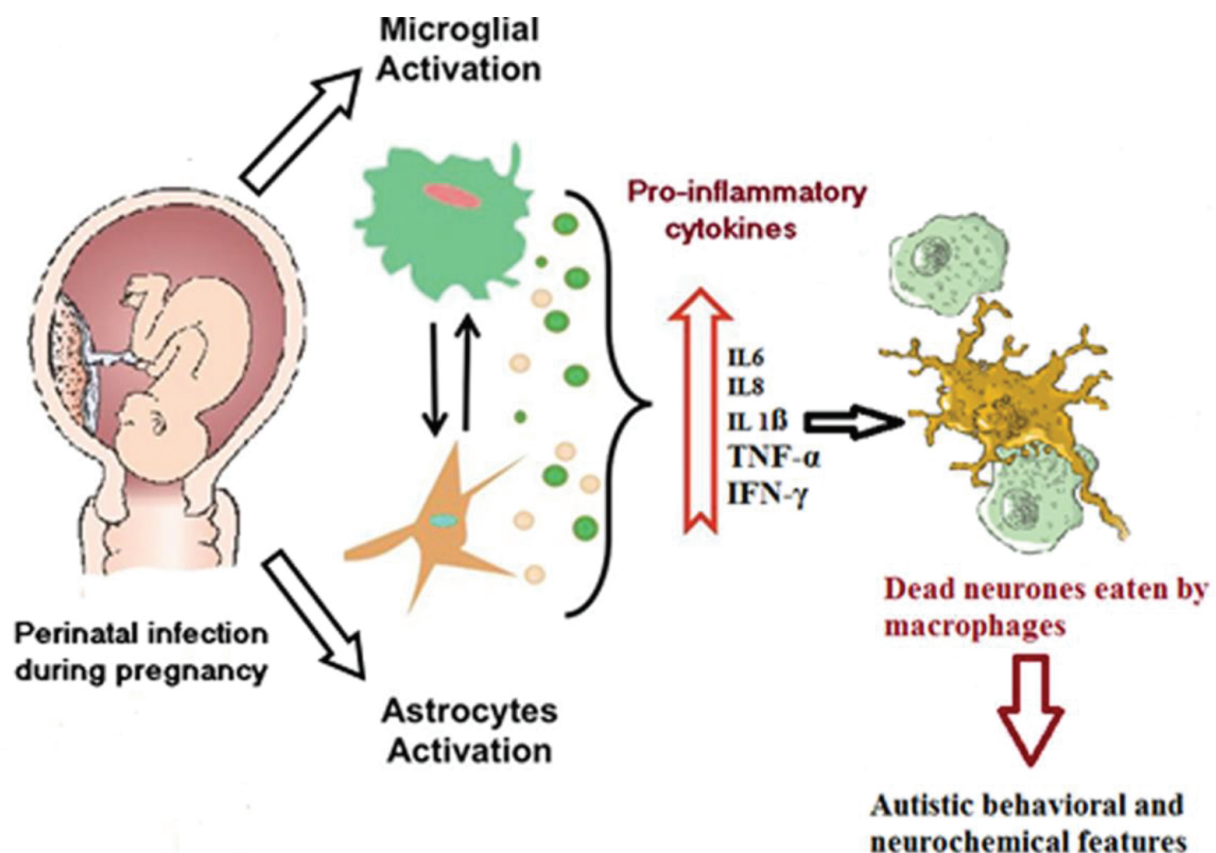
The metabolomic studies of urine from patients with autism have identified molecules associated with the microbiome, such as dimethylamine, hippuric acid, and phenylacetylglutamine [24, 32]. Decreased plasma levels of *p*-hydroxyphenyllactate, the metabolite of *Bifidobacterium* and *Lactobacillus*, which is known to serve as an antioxidant both in the circulation and tissues, were also detected in urine of patients with autism [33].

SCFA, such as acetate, propionate, and butyrate, are neuroactive microbial metabolites that can cross the BBB and induce remarkable changes in brain function during development and thus lead to behavior abnormalities [34–36]. Increased levels of total as well as individual SCFA levels have been associated with autism [37]. Propionate has also been shown to induce behavioral changes similar to autism when infused intraventricularly to the brain [35]. It was also reported that increased butyrate levels in valproic acid (VPA) *in utero*-exposed male offspring could contribute to deficits in social behavior. Both butyrate and VPA neurotoxicity are associated with the impairment of fatty acids transport by the carnithine pathway. This

can easily be related to mitochondrial dysfunction as an etiological mechanism in autism [34]. Both acids can also relate to intestinal inflammatory phenotype common in autistic patients through their inhibitory effects on histone deacetylase in the gut of exposed animals [38]. Modulating intestinal mucus composition through MUC-2 gene expression can affect epithelial protection, gut morphology, and gut microbiota composition [39]. Moreover, if blood butyrate levels are increased, it can affect various neuronal cells directly and thereby affect the maturation of oligodendrocytes and hippocampal neuronal cells in the brain during postnatal development and induce autistic features in treated animals [40, 41].

In the case of neurodevelopmental disorders, such as autism, the neuroimmune system could affect not only function but also brain development [42]. The inflammatory response elicited during pregnancy in the mother can induce inflammation in the fetus through the placenta [43].

Clinical and postmortem studies have shown that neuroinflammatory processes induced during the prenatal period usually remain altered throughout autism pathology. Abnormal inflammatory response to infection of different blood cell populations has been described in autistic children, and such patients usually suffer from chronic gastrointestinal disturbances [24, 37, 44]. It is well known that their mononuclear cells and lymphoblasts produce excessive proinflammatory cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-6, and IL-1 $\beta$ , both basally [45] and after being stimulated with lipopolysaccharides (LPS) [46],



**Figure 4.** Early inflammation as an etiological mechanism in autism.

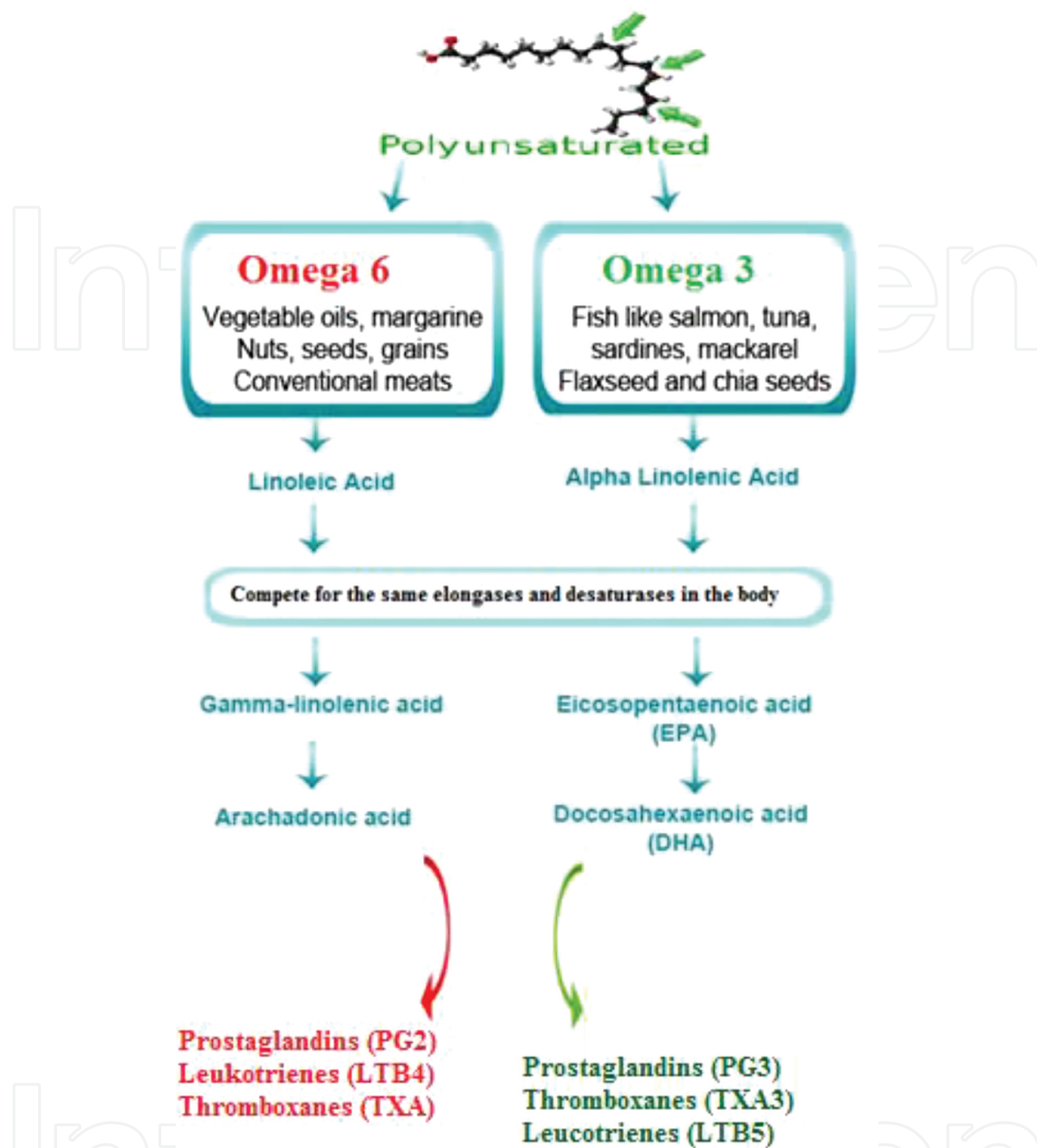


compared to healthy controls. Astrogliosis and microglial activation, together with the overexpression of cytokines in different regions of the autistic brain, show that autistic patients demonstrate an altered neuroinflammatory response throughout their lives; they also show increased astrocyte and microglia inflammatory response in the cortex and the cerebellum [47, 48]. Moreover, increased expression of interferon- $\gamma$  (IFN- $\gamma$ ), monocyte chemoattractant protein-1 (MCP-1), transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), IL-8, IL-6, and TNF- $\alpha$  and other genes associated with the immune response have been reported in those brain regions and in the cerebrospinal fluid [48–50] (**Figure 4**).

In a 2D gel-based proteomic analysis of urine, a total of 250 protein spots were detected in autistic samples compared to 195 in normal control subjects. Whereas 10 proteins were overexpressed, others were found to be underexpressed. Out of the significantly different urine analysis, three overexpressed peptides were identified as kininogen-1 (KNG-1)-50, IgG1 heavy chain variable region, and mannan-binding lectin serine protease-2 isoform-2 precursor-45. The abnormal formation of KNG-1 as an important regulator of urokinase plasminogen activator receptor is involved in cell migration and proliferation [51]. The increase of urinary KNG-1 levels in all the tested autistic children highlights the possibility of using this protein as a diagnostic marker.

Significant changes in the levels of aspartate, citrate, creatinine, hydroxyphenyllactate, indoleacetate, isoleucine, glutamate, and glutarate between autistic and control individuals were identified by West et al. [52]. They identified a decreased level of blood homocitrulline as a new biomarker in autism. Homocitrulline is a poorly understood molecule that is known to be formed inside the mitochondria from lysine and carbamoyl phosphate. The decreased blood level of this marker also suggests that its metabolism in the brain may also be disrupted. Homocitrulline levels are increased in urine and blood in patients with ornithine translocase deficiency, which diverts the reaction between carbamyl phosphate and lysine. Patients with ornithine translocase deficiency exhibit behavioral abnormalities similar to autism, such as developmental delay, spasticity, learning, and cognitive abnormalities, together with frequent seizures [53]. In addition, disrupted brain redox status and energy metabolism were reported in rats that received homocitrulline intraventricularly [54, 55]. These observations suggest that elevated brain levels of homocitrulline are deleterious; however, additional studies are needed to define the brain levels of homocitrulline and its potential role in the development of autism.

The role of polyunsaturated fatty acids (PUFA) in neurodevelopment is becoming clear. The brain and nervous tissue usually depend on  $\omega$ -3 fatty acids, specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), for structural cell signaling purposes [56]. Among the ascertained and reproducible markers in autism are the elevated  $\omega$ -6 PUFA, LA ( $n$ -6) together with  $\omega$ -6: $\omega$ -3 ratio ( $n$ -6: $n$ -3). Elevated  $n$ -6: $n$ -3 ratios above 4–6:1 have been associated with neuroinflammation as an etiopathogenesis mechanism in autism [57]. In several studies, patients with autism exhibited elevated  $n$ -6: $n$ -3 ratio and decreased  $n$ -3 fatty acid compared to intellectually impaired control [58, 59]. Fombonne et al. [60] reported that the absence of incidence of autism from 1991 to 2006 among an Inuit population was attributed to the consumption of large amount of fish.



**Figure 5.** Unbalance between  $\omega$ -6/ $\omega$ -3 fatty acids as etiological mechanism in autism.

Glutamate and GABA as the main excitatory and inhibitory neurotransmitters in the human brain, respectively, have important roles during prenatal or postnatal brain development. Upon excitation of the presynaptic neuron, glutamate is released from the synaptic vesicles into the synaptic cleft. This is followed by the binding of the released glutamate to ionotropic (NMDAR and AMPAR) or metabotropic receptors (mGluR) on the postsynaptic neurons. The binding of glutamate to mGluRs triggers the activation of G-protein-dependent intracellular signaling cascade. The activation of ionotropic glutamate receptors AMPA, kainate, and NMDA by glutamate induces the opening of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  ion channels. The overactivation of glutamate receptors by excessive glutamate results in the influx of high levels of calcium ions

(Ca<sup>2+</sup>) into the postsynaptic cells, which in turn activate a cascade of proteases, lipases, nitric oxide synthase, and a number of enzymes that damage cell structures leading to cell death.

Early studies described the neurotoxic effect of the excitatory neurotransmitter glutamate [61]. In 1969, Olney [62] found that the subcutaneous injection of monosodium glutamate resulted in necrotic brain lesions in the hypothalamus of newborn mice, leading to a number of developmental abnormalities. These reports led to the introduction of the term “excitotoxicity,” describing cell damage induced by an excess of glutamate as the most important excitatory amino acids. The control of extracellular glutamate level at the synapse relatively depends on Na<sup>+</sup>-dependent glutamate transporters located perisynaptically on astrocytes or neurons. Dysfunction of these transporters usually contributed to severe excitotoxicity [63]. It is also true that the activity of glutamate transporters is regulated by the extracellular concentration of glutamate.

Glutamic acid decarboxylase (GAD) as a rate-limiting enzyme in glutamate/GABA cycle catalyzes the conversion of glutamate to GABA. GAD65 and GAD67 are two isoforms of GAD, expressed from two unlinked genes in the adult brain [64]. GAD67 is localized in chromosome 2q31.1, which is related to susceptibility for autism, so it might be a potential biomarker for GABAergic abnormalities demonstrated in autistic patients [65]. The reduction in the number of cerebellar Purkinje cells that express GAD67 abundantly has also been widely reported in autism [66]. A high percentage decrease of mRNA expression for GAD67 was observed in Purkinje cells of autistic individuals compared to control brains [67]. These findings suggest that the reduction in the GABA input to cerebellar nuclei disrupts the output to cerebral cortex and can be related to the motor and cognitive abnormalities seen in autistic patients. An early study has also reported the reduction of GAD65 and GAD67 protein in the parietal cortex and in the cerebellum of autistic brains [68]. There are also reports of increased glutamate levels in the blood and platelets of autistic patients [69, 70]. In addition, a significantly lower glutamate/glutamine ratio was reported by Abu-Shmais et al. [71]. Other studies have also shown that an increased release from presynaptic neurons can also contribute to excitotoxicity. Moreover, increased frequencies and amplitudes of action potential-evoked excitatory synaptic potentials have been observed in mouse models of autism. Therefore, many studies were conducted to clarify and analyze the functional status of glutamatergic and GABAergic neurotransmission in the autistic brain [72, 73]. Based on these studies, strong evidence indicates that dysfunctional excitatory and inhibitory synaptic activities underlie several of the characteristics of autism and support a hyperglutamatergic hypothesis of autism. This was attributed in some studies highlighting an imbalance between GABAergic and glutamatergic as inhibitory and excitatory neurotransmitters, respectively [73]. Another potential source of glutamate excitotoxicity is either the abnormal high release of glutamate or the decrease of glutamate transporters as proteins on the presynaptic neurons and astrocytes playing an important role in the reuptake and removal of glutamate from the synaptic cleft. Raghavendra Rao et al. [74] showed that glutamate transporter-1 (GLT-1) expression was significantly reduced (38–47%) in the rat brain 24 h after fluid percussion injury. The resulting decrease in transport leads to an excess of glutamate in the synaptic cleft. Either increased glutamate release from the presynaptic neuron or removal by glutamate transporters, as two mechanisms that might be related to

glutamate excitotoxicity, has been proven in rodent models of autism [75, 76]. Purcell et al. [77] used 10 brain postmortem samples from individuals with autism to identify genes that were significantly up-regulated or down-regulated. These researchers found abnormalities in the AMPA-type glutamate receptors and glutamate transporters in the cerebellum of autistics compared to control involving these transporters directly in the pathogenesis of this disorder. This was ascertained when the down-regulation of glial glutamate transporters, GLT-1 and GLAST, was found effective in the generation of animal models of autism in which glutamate receptors are overstimulated [76].

Glutamate excitotoxicity can easily be related to low GSH level and this has been repeatedly reported in autistic patients. It is well known that more than 80% of extracellular glutamate is transported into astrocytes [78]. This in turn stimulates both GSH synthesis and cysteine inward flow as a prerequisite for the maintenance of high GSH level in astrocytes [79]. On the contrary, neurons import cysteine through EAAT2 and EAAT3 and this process is competitively inhibited by glutamate. Based on this, glutamate excitotoxicity will starve neurons of cysteine in favor of ensuring high GSH level in astrocytes.

Several reproducible studies have ascertained that individuals with autism demonstrate an abnormal brain 5-HT system [80, 81]. This was clinically presented as hyperserotonemia [80], altered 5-HT synthesis or 5-HT receptor affinity, and dystrophic serotonergic [82–84]. In a recent study on postmortem brains, a significant decrease in both 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> binding was reported in autism [85]. Some researchers suggested an association of 5-HT dysfunction with repetitive and self-injurious behaviors [86–88]. This gives support to the idea that peripheral alterations in the 5-HT system may be an important marker of central abnormalities in autism.

Oxytocin (OT) as a neuropeptide of great interest has been known to play important roles in social behavior in both animals and humans [89, 90]. OT-related studies in autism have repeatedly reported lower blood OT level in autistic patients compared to age- and gender-matched control subjects [12, 81, 91, 92]. The faulty processing of the OT prohormone to the active OT neuropeptide was also found [93] together with abnormalities and polymorphism in the OT receptor (OTR) gene, raising the possibility of OT resistance in autism [94–98]. Multiple intersections of the 5-HT and OT systems have been ascertained and were found to influence behaviors such as sociability, aggression, and anxiety that are relevant to autism [99, 100].

With the move toward the development of disease treatment strategy, there is a great need for more specific diagnostic criteria of autism as a heterogeneous disorder. The diagnostic accuracy of biomarkers is most commonly measured by calculating its sensitivity and specificity. Receiver operating characteristic (ROC) curve analysis is a useful tool in the assessment of biomarker accuracy. The accuracy of a biomarker depends on how well it separates the groups being tested into those with and without the disease in question. In ROC analysis, the ratio of the abnormal found by the marker to the total number of abnormal known to have the disease is the true positive rate (or sensitivity), whereas the ratio of the normal found by the test to the total number of normal is the true negative rate (or specificity). The ROC curve is a graph of sensitivity (*y*-axis) versus 1-specificity (*x*-axis). Accuracy is measured by the area



under the ROC curve. An area of 0.9 to 1 represents a perfect marker, an area of 0.8 to 0.6 represents good-fair marker, and an area of 0.5 represents a worthless marker [101]. More recently, combined ROC was introduced as a simple clinical method with great potential for assisting the diagnosis of autism through the increase of the AUC, specificity, and sensitivity and the diagnostic value of combined markers [102].

### 3. Biomarkers-directed treatment strategies

Positive roles between selected nutraceuticals or vitamins/minerals-based treatment strategies and the improvement of autistic features have been reported. In a comparative case-control treatment strategies, two groups each of 44 autistic patients, with an age range of 2 to 28 years, were given either micronutrient supplement containing (14 vitamins, 16 dietary minerals, 3 amino acids, and 3 antioxidants) without any autism medication or conventional medication without supplementation. Patients in both groups improved, but the level of improvement was remarkably higher in micronutrient recommended group than in the conventional medication group [103].

Similarly, in an observatory study, the administration of vitamin B12 and GSH along with low fructose and food additive/color organic diet of 10 children (4–10 years of age) for 3 to 6 months resulted in a significant improvement in the social interaction, concentration, writing, language, and behavior [104]. A meta-analysis of 18 studies revealed that the supplementation of vitamin B6, especially in combination with magnesium, improved the health of autistic patients [105, 106].

Complementary alternative medicine (CAM) treatments are usually recommended to promote health, to avoid side the effects of conventional drugs, or to ameliorate the core symptoms of autism. Prenatal exposure to VPA induces a rearrangement of early microbial colonization, leading to an increase of butyrate levels in the gastrointestinal tract of male offspring. Consequently, increased levels of butyrate in the gut may interfere directly with gene expression in intestinal cells or indirectly with gene expression of neuronal cells after crossing the BBB. This is usually accompanied with induced deleterious changes in the intestinal and brain functions that might explain certain autistic features. These results open the road to a novel strategy to treat autism through gut microbiome manipulation. The administration of probiotic or prebiotic may provide an excellent tool to treat autism based on our understanding of the role of gut-brain axis and microbial metabolites in the pathology of this disorder.

Clinical studies have shown that prenatal supplementation with *n*-3 PUFA may be beneficial for healthy neural development in both preterm and full-term infants [107–109]. Regarding preterm infants, the timing of supplementation is of critical importance because these infants cannot fully use accumulated long-chain PUFA that usually start in the last trimester of gestation. Studies in full-term infants show that both prenatal and postnatal supplementations cause an improvement in cognition [110, 111]. Moreover, in humans, the outcome of infant *n*-3 PUFA supplementation on long-term brain development appears to be subtle [112–114] compared to the rodent model studies [115], which demonstrate more pronounced beneficial



effects of *n*-3 PUFA supplementation [115–117]. Overall, our adequate dietary *n*-3 PUFA levels starting early in life may support optimal neural development in healthy full-term infants.

In a randomized, double-blind, placebo-controlled 6-week pilot study, Amminger et al. [118] investigated the effects of 1.5 g/d  $\omega$ -3 fatty acids (0.84 g/d EPA and 0.7 g/d DHA) supplementation given in the form of seven pale-yellow, 1 g gelatin capsules of fish oil, each containing 120 mg EPA and 100 mg DHA plus 1 mg vitamin E to 13 autistic children (aged 5–17 years). The placebo was seven gelatin capsules of coconut oil that were of similar shape and size and also contained 1 mg vitamin E as well as 1 mg fish oil to mimic fish taste. The experimental daily dose of 1.5 g  $\omega$ -3 fatty acids is based on a study in children with developmental coordination disorder by Richardson and Montgomery [119]. The findings of their trial suggested that  $\omega$ -3 fatty acids may be effective in treating the aggression and impulsivity in autistic patients. The underlying mechanism of action is not fully understood but may be related to the modulation of serotonergic and dopaminergic neurotransmission [120]. This can be ascertained by considering the phenomenon that DHA or the EPA/ARA ratio might control aggression by depressing the noradrenergic system [112]. The more recent work of El-Ansary et al. [116] supports that the essential fatty acids/long-chain PUFA and  $\omega$ -3/ $\omega$ -6 ratios, phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine could be used as potential biomarkers that point to specific mechanisms in the development of autism and may help tailor treatment or prevention strategies (**Figure 5**). In the recent study of Weiser et al. [121], DHA supplementation greatly reduced the level of IL-6 as an acute inflammatory marker induced in the rodent model of autism exposed to the viral mimetic polyriboinosinic-polyribocytidylic acid during gestation. This gives preliminary evidence that  $\omega$ -3 fatty acids may be an effective treatment strategy for children with autism.

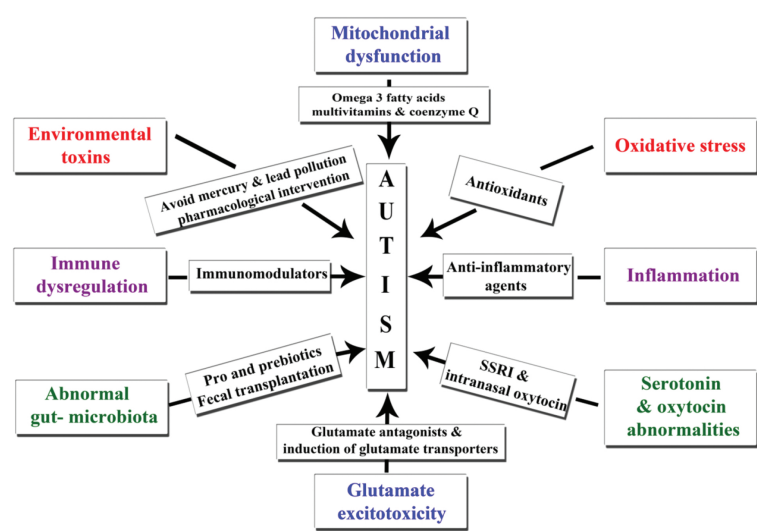
A recent report by the Autism Genome Project Consortium identified a new linkage peak for autism in the region of chromosome 11 where the gene for EAAT2 is located [122]. Signs of astroglial, oligodendroglial, and microglial dysfunction were reported in the autistic brain, suggesting that all these cellular processes may represent presumptive targets for novel therapeutic strategies [123]. Additionally, a study investigating the effects of ceftriaxone and cefixime, activators of GLT-1, demonstrated that these drugs improved some symptoms of autism and decreased epilepsy seizures by increasing the expression of the GLT-1, which reduces extracellular glutamate levels [124].

Elevated levels of glutamate are present in high proteins, including wheat gluten and milk casein. This can explain the sensitivity of autistic children to both proteins as a rich source of glutamate. Some parents of autistic children try gluten/casein-restricted diet in an attempt to improve their child's behavior, but evidence is lacking that following such diet improves the child's challenging behaviors, cognitive and social functioning as core symptoms of autism [125]. Furthermore, following a gluten casein-free diet may place children at risk for suboptimal bone development [126].

Several animal models and research in typically developing volunteers suggest that the manipulation of the OT system may have a potential therapeutic effect in treating social deficits in autistic patients. Twelve weeks of intranasal treatment with OT (0.4 IU/kg/dose) was found effective in improving social impairment, repetitive behavior, and anxiety in children and

adolescents with autism. Some measures suggest the safety and maintenance of the effect for 3 months after the discontinuation of intranasal OT treatment [127]. Meziane et al. [128] reported that an early OT treatment just after birth could be a novel therapeutic approach for the treatment of autism.

In a recent study, Carminati et al. [129] tested the therapeutic efficacy of venlafaxine, an antidepressant drug that inhibits the reuptake of 5-HT, and proved that venlafaxine at a low dose represents a substantial improvement in repetitive behaviors, restricted interests, social impairment, communication, and language. Venlafaxine probably acts via serotonergic mechanisms by affecting the selective 5-HT reuptake inhibitors. **Figure 6** summarizes the biomarkers directed to treat autism.



**Figure 6.** Biomarkers-directed strategies to treat autism.

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