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The relative usefulness of the identification and analysis of biomarkers for the diagnosis of autism spectrum disorders in early childhood and the implementation of personalized precision medicine

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Abstract

Despite progress towards understanding the etiological mechanisms of autism spectrum disorder (ASD), efficient treatment strategy for the primary clinically presented autistic features remain elusive. Up to the heterogeneity and complex nature of ASD, clinical presentation differ in severity and usually accompanied with multiple comorbidities, including gastrointestinal (GI) problems, sleep disturbances, epilepsy, and attention deficit hyperactivity disorder (ADHD). Discovery of biomarkers of ASD is essential not only for clarifying the clinical features of this disorder but also as an primary diagnostic implement that could help to tailor early interventions. Therefore, this review describes the core areas of ASD biomarker research, including GABAergic/glutamatergic imbalance, mitochondrial dysfunction, hyperserotonemia, and impaired gut microbiota, all of which have demonstrated success in diagnosing ASD and could serve as targets for implementing personalized precision medicine. Additionally, this review includes accomplishment that focus on the importance of precision medicine and the current trials that make use of ASD biomarkers detection.

Keywords: Autism Spectrum Disorders; Biomarkers; Personalized precision medicine; GABA; Glutamate; Mitochondrial dysfunction; Gut microbiota.

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1. Introduction

Autism spectrum disorder (ASD) as a neurodevelopmental disorder is clinically presents as persistent impairments in social interaction and repetitive forms of behavior or reactions that existing during the early development of child's brain (APA, 2013). In children with ASD, early intervention may moderate the severity of autistic phenotypes. Approximately 25% of children with early diagnoses could reach satisfactory level of social, adaptive, and cognitive skills comparable to optimal outcome (Sutera et al., 2007; Helt et al., 2008; Fein et al., 2013). Interestingly, a randomized clinical trial examining the effects of an early behavioral intervention on children ≤ 30 months diagnosed with ASD found no significant variances between the intervention and control groups immediately; but, after a 2-year follow-up, the intervention group showed remarkable perfections in the core features and adaptive behaviors of ASD compared to a healthy neurotypical group (Estes et al., 2015). In a recent meta-analysis review based on 35 studies, 55 cohorts with 66,966 individuals with ASD from 35 countries the mean age at diagnosis is 60.48 months (van 't Hof et al., 2021). However, the current practice is to identify ASD as early as possible so that interventions may be implemented earlier, which could lead to better outcomes (Reichow, 2012; Koegel et al., 2014; Sicherman et al., 2021; Pierce et al., 2021).

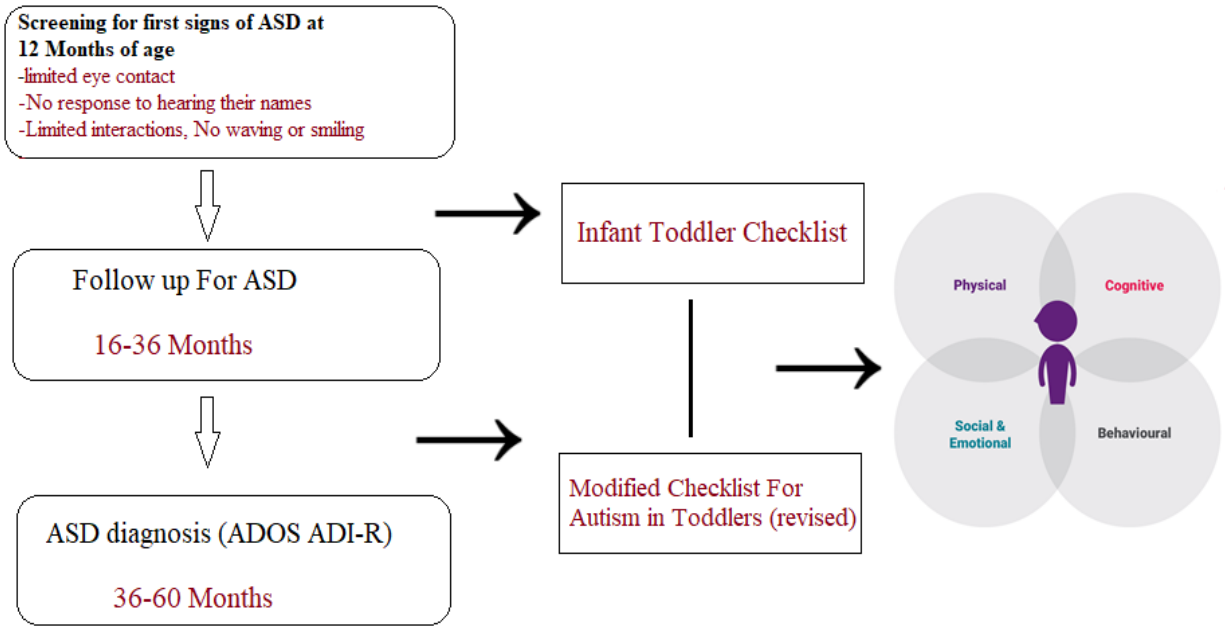

Figure 1: Schematic representation of early detection and intervention strategies in ASD.

2. Challenges for ASD diagnoses and interventions

ASD symptoms usually begin to appear by 3 years of age. Although clinical presentation of ASD may not wholly noticeable till school admition age or after, research has suggested that symptoms can appear between six and eighteen months of age (Szatmari et al., 2016). There is a link between early observed abnormalities, associated medical conditions, and severity of cognitive impairment. Screening usually offers a consistent practice to ensure that children are thoroughly monitored for early signs of ASD in order to promote earlier diagnosis and intervention. Screening of children aged 18 to 24 months can assist in early detection. It is easy to recognize severe cases at earlier ages as compared to mild - moderate cases (Zwaigenbaum et al., 2016). The formal diagnosis of ASD requires clinical proficiency and meeting an extensive set of "gold standard" criteria, which can

substantially reduce early identification of ASD. Difficulties in accessing diagnostic tools, intervention facilities, and training experts and specialists, are global challenges in the diagnosis of ASD and can increase the age of diagnosis. The diagnosis of ASD is required for proper therapeutic and intervention plans. In addition, the success of clinical trials that examined specific treatments and outcomes also demonstrated another challenge: the application of consistent and reproducible measures of assessing improvement. Current approaches depend chiefly on behavioral tests that are biased, less consistent, and greatly affected by the "placebo effect." Gabis et al. (2021) hypothesized that some of the unsuccessful pharmacological and behavioral treatment trials are mostly attributed to the combination of biological and behavioral measures (which are strongly affected by the placebo response) that are utilized as endpoints for the studies. It is possible that some of the drugs or behavioral interventions would have resulted in further improvements by using a subgroup of ASD patients that were more biologically homogenous and a more consistent and objective biomarker for assessing improvements.

Impaired social interaction and communication as well as restricted and repetitive behaviors are the main autistic features. ASD cases are predominantly male, with nearly four males diagnosed with ASD for each female diagnosed; however, the sex ratio seems to decline with severity (Werling and Geschwind, 2013). While this noticeable sex difference is found in all studied populations, variance in the clinical presentation in females, and probable associated diagnostic biases, require additional investigation (Dworzynski et al., 2012). A range of comorbid medical conditions are commonly associated with ASD, such as intellectual disability, sensory hyper or hypo sensitivities, dysibiosis, immune response insufficiencies, epilepsy, anxiety, and sleep difficulties (Croen et al., 2015, Matson and Cervantes, 2014).

The National Institute of Mental Health (NIMH) Research Domain Criteria (RDoC) initiative was developed to motivate investigators to evaluate diagnostic tools and give more attention for behavioral and neurobiological features that could be targeted to improve our understanding of typical, versus pathological, features (Cuthbert and Insel, 2013). Tools have been established to measure the range of behavioral dimensions characteristic of ASD (e.g., the Social Responsiveness Scale (SRS) and the Childhood Autism Rating Scale (CARS)), and such assessments are being used more widely. Approaches for creating continuous severity scores among individuals diagnosed with ASD have also been developed (Hus et al., 2014).

3. The relationship between the neurobiological and neurochemical bases of ASD and altered behavior

In the last decade, social neuroscience as field of research has stressed the contribution of neuroanatomical brain regions in the impairment of social behavior, as core features of ASD clinical presentation (Tomalski et al., 2009; Wolff and Piven, 2013; Dinstein, et al., 2011). This relationship seems to be bidirectional. That is, the ability to show appropriate social behavior skills (i.e., suitable eye contact) affects neurobiological measures. In turn, neurophysiological, neuroanatomical, and neurochemical actions happening in cortical and subcortical brain regions initiate and control an individual's capability to perceive meaning in rational traits of communication. These skills include interpreting facial expressions, proper use of eye contact, and mutual responsiveness (Sullivan et al., 2007; Lombardo et al., 2014). Most recently, abnormal Cerebellar Development is increasingly thought to contribute to aberrant social and linguistic functions which are hallmarks of ASD (van der Heijden et al., 2021).

Many neurochemical pathways contribute to the etiology of ASD; nevertheless, it remains unclear how these complexes signaling interact and induce the core symptoms of autism. Despite the large number of studies on neurochemical alterations in autism, additional studies are essential to shed more light on the etiological mechanisms that contribute to the initial neurodevelopmental differences that lead to the substantial heterogeneity of ASD and thus indicate novel strategies for the prevention and treatment of autism through personalized precision medicine.

Neurochemical dysfunctions of numerous neuropeptides and neurotransmitters, including oxytocin, vasopressin, melatonin, vitamin D, opioids, GABA, glutamate, serotonin, dopamine, and acetylcholine, contribute to the etiology of autism. Recently, Sacai et al. (2020) suggested that altered neurotransmission has a central role in the etiology of

ASD. The excitatory/inhibitory imbalance theory, which involves the synaptopathy of the GABAergic and glutamatergic systems which is related to the impaired social behavior as the core symptom in ASD, and has been validated through the long duration depolarization of neuronal cells in the medial prefrontal cortex of mice. The imbalanced inhibitory/ excitatory neurotransmission induces a marked impairment in social interaction and handling of information (Yizhar et al., 2011; Zhaoqi et al., 2020). In a translational magnetic resonance spectroscopy [1H]MRS studies on humans with autism and in five mouse models and one rat model of ASD, idiopathic adult ASD recorded lower glutamate levels in the striatum, high affinity GABA transporters, than controls. Mouse models included: mice prenatally exposed to VPA through their mothers, BTBR T+tf/J mice, 15q11-13 patDP mice Shank3 KO mice, Nlgn3R451C KI mice C57BL/6J genetic, and in addition, Nlgn3 KO rats (Horder et al., 2018; Laura et al., 2019; Puts et al., 2017).

Several studies have highlighted a relationship between single-nucleotide polymorphisms (SNPs) of GABAA receptors positioned on chromosomes 15q11–q13 and individuals with ASD (Buxbaum et al., 2002; Kim et al., 2006). However, a latest meta-analysis revealed that SNPs of B3, A5, and G3 subunits of GABAA receptor were not associated with ASD in many populations (Mahdavi et al., 2018). This contradict could be explained on the basis that increased GABA production in ASD could compensate for GABA receptor dysfunction (Yip et al., 2008). Furthermore, Mahdavi et al (2018) research was not based on GABAB receptor dysfunction which have been proved in many studies with ASD (Silverman et al., 2015; Huang et al., 2022; Jiang et al., 2022).

Surprisingly, lower excitatory synaptic transmission and much higher I/E ratio in pyramidal neurons of the developing mouse prefrontal cortex (PFC) lead to impaired social interaction as well as speech abnormalities, which may trigger the etiolopathology of a subgroup of autistic patients. Thus, improvement of excitatory synaptic transmission might be promising for treating particular ASD patients. This might suggest that imbalances in E/I neurotransmission should be targeted by personalized precision strategies (Sacai et al., 2020).

El-Ansary (2020) suggested a medical hypothesis through which reversal of imbalanced GABAergic/glutamatergic neurotransmission could be used as a strategy for personalized treatment. This hypothesis integrates the therapy to restore normal GABAergic signaling through GABA supplementation with antioxidant status, vitamin D status, intracellular chloride concentration [Cl−]i, normal gut microbiota, functional glutamate transporters, and active GABA receptors.

It is well accepted that insufficiency or deficiency of vitamin D are usually accompanied with much lower activity of glutamic acid decarboxylase, the rate limiting enzyme in GABAergic interneurons together with lower levels of glutamate and glutamine in mouse brain tissue (Groves et al. 2013). This suggests that impaired glutamate-glutamine-GABA cycle as a consequence of glutamate excitotoxicity could be corrected through vitamin D supplementation (Vinkhuyzen et al., 2018). In relation to ASD patients,Wang et al. (2021) reported a significantly much lower vitamin D levels in individuals with autism compared to healthy controls. Vitamin D deficiency was positively correlated with the severity of autistic features. Since vitamin D deficiency is associated with the high incidence of ASD, regular measurement of vitamin D levels in children and adolescents with autism could be a critical precision strategy to correct the imbalance in E/I neurotransmission. Additionally, because pregnant and lactating women consume more vitamin D than usual (yet are usually vitamin D insuffecient), vitamin D status should be recommended as routine test during pregnancy and lactation to provide proper clinical intervention at the proper time.

Developmentally controlled intracellular chloride concentrations ([Cl−]i) are the main factor that contributes to GABA action at GABAA receptors. In immature neurons, GABA signaling mostly induces depolarizing response in the presence of high [Cl−]i, which plays an active role in neurogenesis and synaptic networks; however, in mature neurons, a hyperpolarizing effect of GABA signaling usually occurs under low [Cl−]i concentrations (Watanabe and Fukuda, 2015). The K+-Cl− cotransporter KCC2 facilitates the efflux of Cl− from cells (Rivera et al., 1999), and the Na+, K+-2 Cl− cotransporter NKCC1 stimulates Cl− uptake (Yamada et al., 2004). The reduction in [Cl−]i, which take place during the maturation of neural cells is crucial, resulting in a shift

of GABAergic transmission from excitatory to inhibitory, and is under transcriptional and epigenetic control (Yeo et al., 2013). Bai et al. (2018) reported that oxidative stress or poor antioxidant status caused cotransporter KCC2, resulting in much higher [Cl−]i. By treating oxidative processes and scavenging ROS, normal levels of KCC2 and [Cl−]i can be restored (Abruzzo et al., 2021). This suggests multiple integrated strategies for treating autistic patients should be considered from a personalized precision perspective. For example, using GABA supplementation to treat autistic individuals suffering from vitamin D deficiency, imbalanced antioxidant/pro-oxidant status, or high [Cl−]i will be ineffective.

the GABA neurotransmitter to lose its inhibitory effect and that this dysfunction was concomitant with a marked decrease in the expression of the chloride

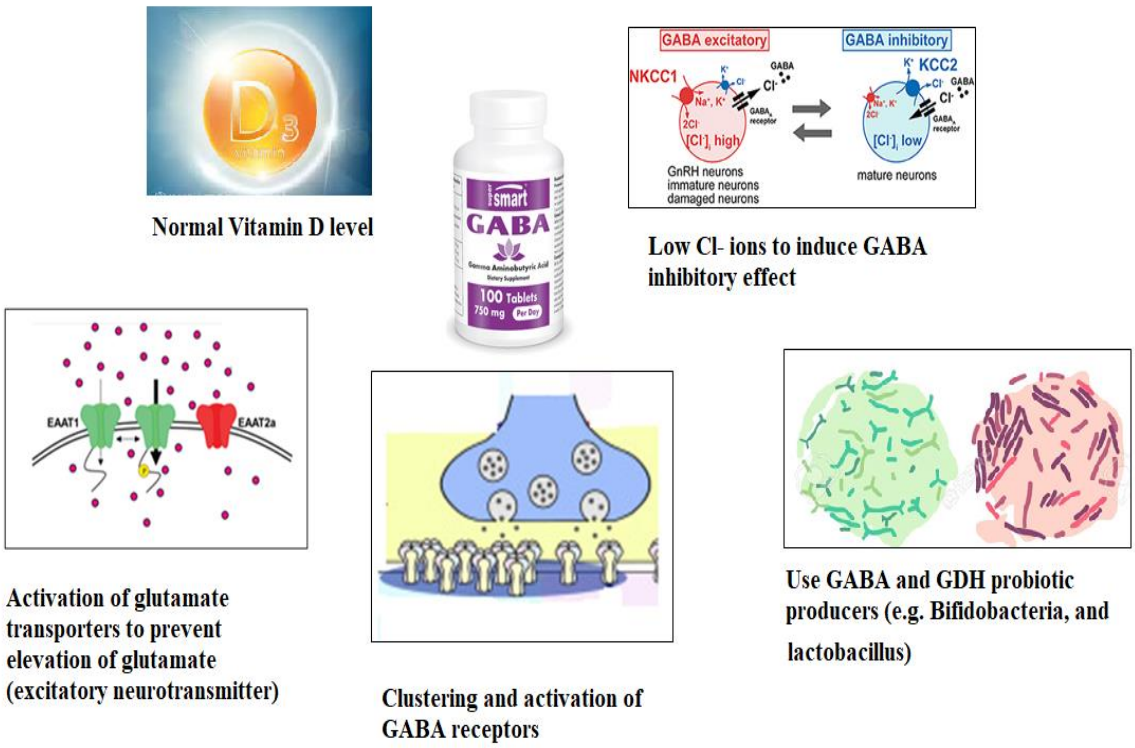

Figure 2: Integrative factors for a precision strategy that successfully treats glutamate excitotoxicity in ASD patients with GABA supplementation.

4. Biomarker–directed personalized precision treatment strategies for ASD

The aim of precision medicine is to offer the correct treatment at the right time for each patient. Biomarkers are generally defined as a specific variable that is an indicator of normal biological or pathological processes or pharmacological responses to a therapeutic intervention (Atkinson et al., 2013). Biomarkers of ASD may be detected before birth and after diagnosis, and some are expected to respond to precision medicine. Many highly predictive biomarkers of ASD have been detected. Nevertheless, most of these biomarkers are preliminary and their role in the detection and arly intervention of ASD prerequisites further investigation (Frye et al., 2014). Combining known biomarkers would probably be more effective in the early identification of ASD and thus could guide successful treatments. Biomarkers include measurements of brain function or anatomy (electroencephalogram [EEG], imaging), or proteomic and metabolomics candidates that indicate alterations

in immune function, oxidative stress, or neurotransmission. In addition, clinical presentation that are established by direct examination of patients could also be used as markers. Medical (e.g., epilepsy, sleep troubles, altered gut microbiota) and psychiatric features among which is abnormal sensory sensitivity (i.e hypersensitivity/hyposensitivity) may represent appropriate treatment targets.

The current drive for precision medicine stems from the lack of effective early intervention or treatment strategies and the need to determine the phenotypic and etiological inconsistencies between individuals. This drive aims to target treatments based on understanding of the etiopathological mechanisms of ASD and to combine interventions (or drugs) with diagnostic biomarkers to recruit or exclude patients to precisely manage this disorder. Among these etiological mechanisms are glutamate excitotoxicity, oxidative stress, mitochondrial dysfunction, neuroinflammation, Altered gut microbiota, and heavy metal toxicity. Figure 3 demonstrates different molecular etiological mechanisms of ASD.

comorbidities can guide pharmacological control strategies. Other markers more specific to ASD have a less clearly defined relationship to treatment. Defined autistic features such as impairment in social communication, repetitive behaviors, and linked

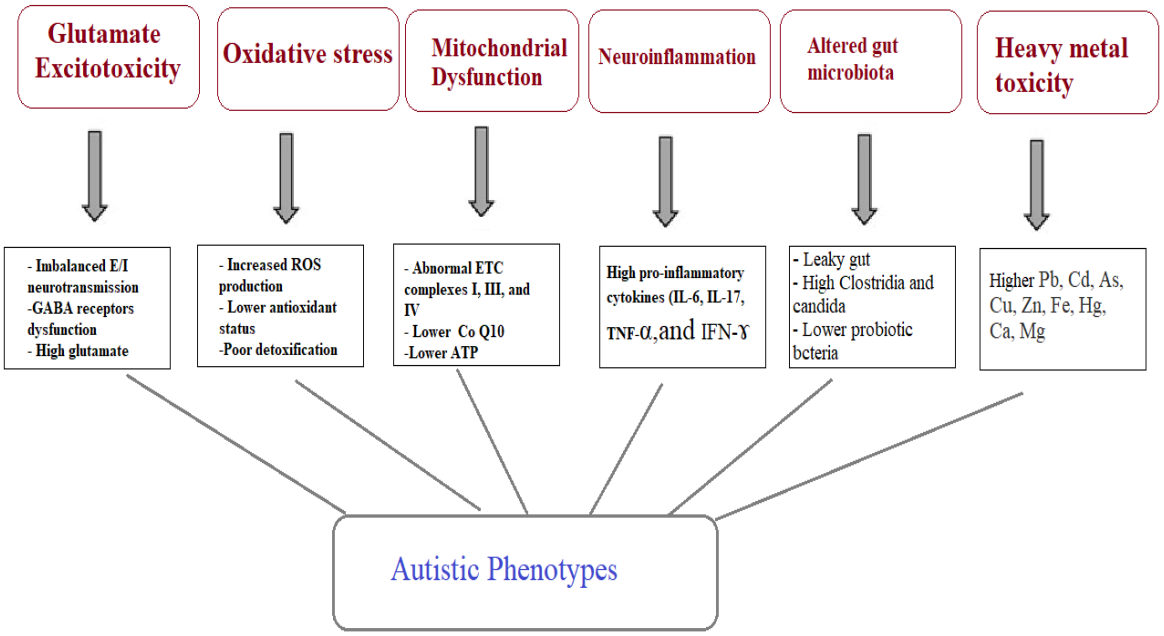

Figure 3: Different molecular etiological mechanisms of ASD and related biomarkers.

The main advances in drug treatment of ASD originated from the identification of highly penetrant genes in individuals with autism. Hundreds of ASD risk genes have been identified (Betancur, 2011; Bourgeron, 2015), and more are anticipated to be identified in the future through larger sample sizes and sequencing of whole the genome. The impact of these discoveries lies in their capability to find a probable fundamental link between a gene and principal cellular signaling pathways that are related to the clinical presentation of autism (Spooren, 2012). Based on this link, specific biological processes, such as oxidative stress, neuroinflammation, receptor signaling, and glutamate excitotoxicity, could be targeted rather than individual gene products, and these intervention methods could apply to larger patient groups with similar clinical presentations.

Multimodal biomarkers such as the combination of resting-state EEG and fMRI, may have better prognostic and predictive value when compared to single modality biomarkers. For example, deviations in gamma band oscillations possibly demonstrate either higher excitatory (e.g., glutamatergic) or lower inhibitory (e.g., GABAergic) signaling. More information on glutamate and GABA levels resulting from MRS may assist in the understanding and interpretation of EEG scores. It would be highly useful to specify whether a glutamate (receptor) antagonist, or GABA agonist would be highly recommended for a given individual. Additionally, a clear understanding and assessment of a patient's cognitive ability across different domains is essential for ASD individuals and their families and caregivers to agree to future medical interventions. Longitudinal designs with no less than three-time intervals that simultaneously measure alterations in clinical presentation and neurocognitive, functional, and anatomical courses to find out the prognostic value of biomarkers are desirable. As large clinical trials have tried to target GABAergic (arbaclofen) and glutamatergic (memantine) systems, markers representing the activity of both systems, as evaluated by MRS or other proxy markers such as EEG gamma band activity, would be particularly useful (Rojas et al., 2014).

Additional markers that may be appropriate for early intervention could include immune system-related biomarkers that frequently affected in ASD (Ashwood et al., 2006; Onore et al., 2012), such as whole-blood serotonin (Anderson et al., 1990; Cook, 1990), genetic polymorphisms that influence serotonergic transporters (Sutcliffe et al., 2005), or serotonin ligand markers on positron emission tomography (Chugani et al., 1999; Murphy et al., 2006; Chugani et al., 2008; Goldberg et al., 2009; Beversdorf et al., 2012). In the context of personalized precision medicine, understanding the persistent changes in 5-HT signaling in ASD could be a critical prerequisite for successful treatments with selective serotonin reuptake inhibitors (SSRIs). SSRIs block 5-HT reuptake through pharmacologic inhibition of the serotonin transporter, increasing extracellular levels of 5-HT. Improved extracellular levels of 5-HT with SSRIs mends symptoms in some individuals with ASD (Hollander et al., 2012) while it is not useful in others. For example, only approximately 35% of children and adolescents (Hollander et al., 2012) with ASD demonstrate lower irritability, repetitive behavior, and inappropriate communication and hyperactivity when receiving SSRI treatment (Fatemi et al., 1998; Hollander et al., 2012; Williams et al., 2013; Taylor et al., 2012). While the reason for this difference in response is still unclear, one potential explanation of the inadequate or absent therapeutic efficiency of SSRIs in some individuals with ASD is that these patients already have elevated extracellular 5-HT (Sugie et al., 2005). For example, these nonresponders could have hypofunctional serotonin transporter (SERT) gene variants and consequently have higher 5-HT levels extracellularly, making additional block from medications useless.

Approximately 5% of individuals with ASD have a mitochondrial disease (Rossignol and Frye, 2012), and 30–80% display some degree of mitochondrial dysfunction (Mtd) (Rose et al., 2018). Some blood variables, such as ammonia, aspartate aminotransferase (AST), and creatine kinase, are commonly used biomarkers for identifying mitochondrial dysfunction in ASD, but some of them require more studies to be confirmed (Rossignol and Frye, 2012). Lactate dehydrogenase, lactate oxidase, pyruvate kinase and hexokinase, Na+/K+ ATPase, Caspase-3 and Caspase-7 are biomarkers related to mitochondrial dysfunction that can help discriminate between ASD patients and healthy controls (Al-Al-Mosalem et al., 2009; Khemakhem et al., 2017; El-Ansary et al., 2010; 2011 &2017).

Overall, identification of mitochondrial dysfunctions as an etiological mechanism in individuals with ASD is very promising from the precision medicine perspective since these children may respond differently to specific treatments depending on the type of dysfunction (Frye et al., 2013). Elevations in levels of proteins, lactate, pyruvate and even white blood cells were confirmed in the cerebrospinal fluid of patients with mitochondrial dysfunction and individuals with ASD (Zecavati and Spence, 2009). In addition, MtD and oxidative stress may clarify the known 4:1 male/female ratio in autism due to increased susceptibility of males to both dysfunctions. Biomarkers related to mitochondrial dysfunction have been recorded, but they appear generally underutilized regardless of the available treatment interventions (Poling et al., 2006).

From a clinical perspective, CoQ10 deficiency is well-recognized and could be both primary and

secondary (Quinzii and Hirano, 2011). In the primary group, biallelic mutations in the genes encoding CoQ10 synthetic pathway enzymes result in significantly diminished levels of CoQ10 concomitant with a diversity of clinical presentations; therefore, supplementation with CoQ10 may be effective in these patients (Emmanuele et al., 2012). Deficiency of CoQ10 has also been recorded in a large number of patients with respiratory chain deficits, but supplementation with CoQ10 in this group has been of inadequate usefulness.

Delhey et al. (2017) showed promising results from activating complex I, complex IV, and citrate synthase by administrating selected mitochondrial supplements, such as coenzyme Q, fatty acids, folate, and vitamin B12. Therefore, understanding the etiological mechanisms of mitochondrial dysfunction in ASD could be essential for identifying new therapeutic targets of autistic features. A recent study evaluated mitochondrial energy generation in a BTBR rodent model of ASD (Ahn et al., 2020). The authors examined alterations in mitochondrial morphology, which can directly influence mitochondrial bioenergetics-related function. They reported that BTBR mice had abnormal mitochondrial function demonstrating more scrappy mitochondria than C57BL/6J healthy controls. Consistent with the increase in mitochondrial fragmentation, they found that two fission proteins, pDRP1S616 and pMFFS146, were activated and phosphorylated in BTBR mice. In addition, Frye et al. (2021) studied electron transport chain (ETC) complex activity and mitochondrial morphology measurements in fibroblasts obtained from 18 individuals with ASD and 4 healthy controls. In the ASD patients, symptom severity was measured by the SRS and Aberrant Behavior Checklist (ABC). Mixed-model regressions indicated that abnormal mitochondrial morphology were concomitant with the activity of ETC complexes I, III and IV. When compared with individuals with classic mitochondrial illnesses where ETC activity is much lower, some patients with ASD have heightened ETC activity. An increase in ETC complex IV activity was first described in ASD through muscle biopsy (Frye et al., 2011) and later ascertained in other tissues, such as fibroblasts (Frye et al., 2013), buccal epithelium (Frye et al., 2016), brain (Palmieri et al., 2010) and lymphoblastoid cell lines (LCLs) [36]. Since ETC complex IV is the mitochondrial complex responsible for oxygen consumption, increases in ETC complex IV activity lead to markedly higher oxygen consumption or increased mitochondrial respiration. An LCL model in a subset of ASD individuals found a 200% elevation of mitochondrial respiration compared to control LCLs (Frye et al., 2016; Hassan et al., 2020; Frye et al., 2017; Rose et al., 2014, 2015 a, b & c, Bennuri et al., 2019; Rose et al., 2017 &2018).

Most ETC complexes (I, III, and IV) transport protons across the inner mitochondrial membrane to produce a proton gradient that drives ETC complex V, also known as ATP synthase, to produce ATP. The ETC is the major source and target of reactive oxygen species (ROS), such as oxygen radicals. Oxidative stress occurs when the amount of ROS become increased to the point that they affect normal ETC function. To decrease ROS, mitochondria leak protons back across the inner mitochondrial membrane, essentially reducing the proton gradient and decreasing mitochondrial efficiency. Interestingly, LCLs from individuals with ASD with higher respiratory rates are associated with increased proton leakage, uncoupling of the respiratory chain and superoxide elevation in the mitochondrial compartment (Rose et al., 2014). Consistent with this finding, ETC complex I and IV activity were relatively dissociated in buccal epithelium from individuals with ASD, suggesting an uncoupling of the respiratory chain (Delhey et al., 2017; Goldenthal et al., 2015).

Much like mitochondrial malfunction in ASD, neurogenesis may be severely impacted as well. Neurons in the central nervous system require the most energy out of all cell types. Most of the ATP produced in the central nervous system is typically utilised to preserve synapse function and neuronal excitability.

To maximize local energy release, mitochondria can be attached to dendrites and synapses to produce ATP, thus stimulating vesicle recycling during neuronal action.48 However, in addition to postmitotic neurons, mitochondrial oxidative phosphorylation might also play a critical role during neurogenesis. Taken together, mitochondrial dynamic dysfunctions and alterations in mitochondrial morphology or impaired ETC oxidative phosphorylation bioenergetics could have profound effects on physiological neurogenesis and on the proper establishment of neuronal function in the brains of ASD patients.

Figure 4 illustrates the role of mitochondria in neurogenesis, synaptogenesis, and synaptic plasticity and how impairment of these physiological processes could be related to ASD.

A ketogenic diet (KD), which is a high-fat, low-carbohydrate and low-protein diet, is known to reduce autistic behaviors in both humans and rodent models of ASD. Interestingly, supplementation of BTBR mice treatment as a form of personalized precision medicine.

From the bioenergetics perspective, a KD has a positive effect on energy metabolism. For example, it increases the levels of adenosine triphosphate (ATP) and enzymes associated with mitochondrial energy generation (Bough et al., 2006; Gano et al., 2014; Marti, 2014). Acetyl-CoA, a product of fatty acid oxidation, is transformed to ketone bodies through the catalytic activities of D-β-hydroxybutyrate dehydrogenase, acetoacetate succinyl-CoA transferase, and acetoacetyl-CoA-thiolase enzymes (Hartman et al., 2007). Ketone bodies, such as β-hydroxybutyrate, acetoacetate, and acetone, serve as energy compounds in case of stravation, and are capable to cross the blood–brain barrier (BBB) to nourish the brain (Figure 5).

with a KD amends both mitochondrial function and morphology. Furthermore, a KD decreased pDRP1S616 levels in BTBR mice, probably involved in the repair of mitochondrial morphology. These data provide additional proof that impaired mitochondrial energy-generation related functions and mitochondrial fragmentation may be considered as etiological mechanisms of ASD which can be reversed with KD

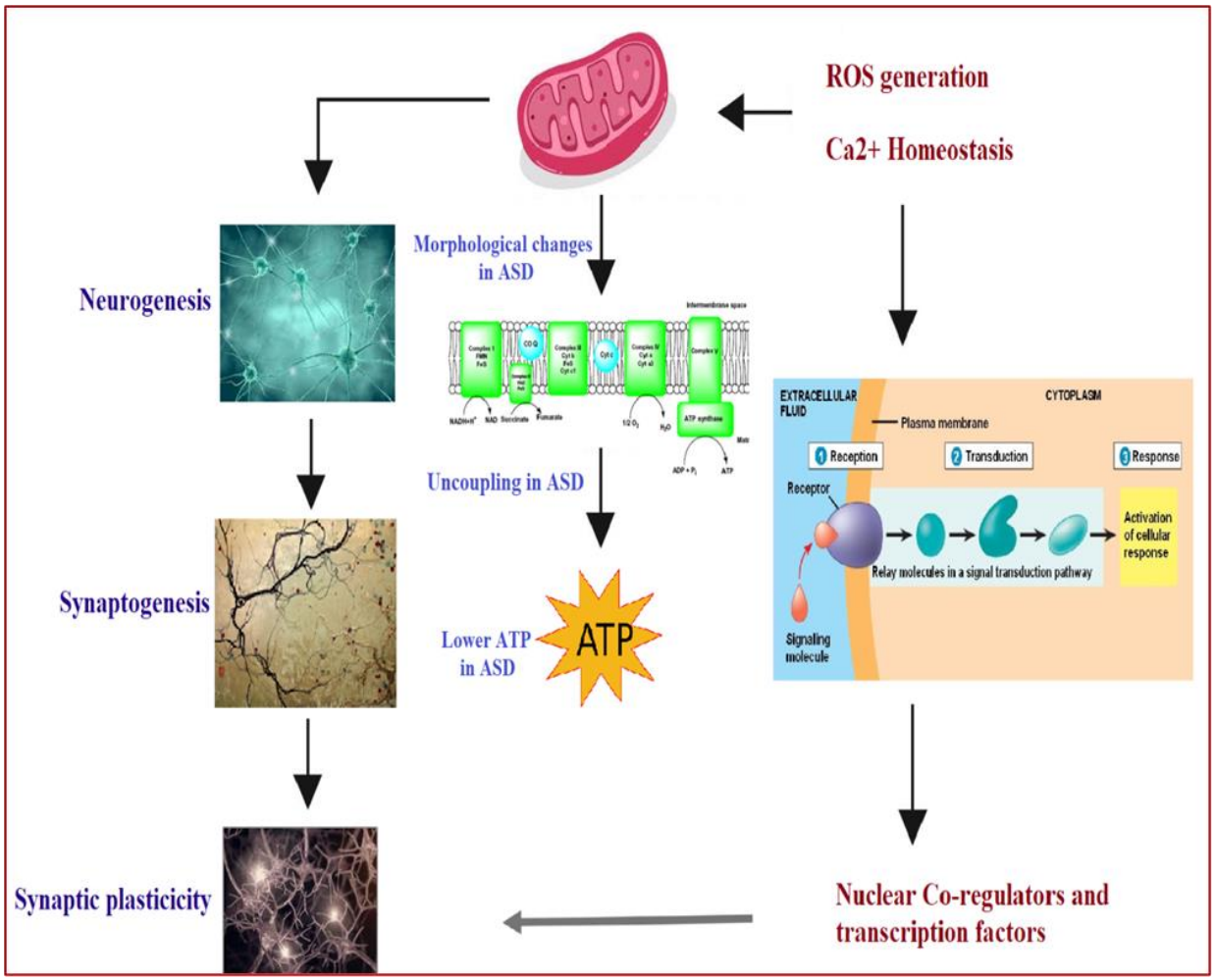

Figure 4: The role of changes in mitochondrial morphology, uncoupling of respiratory complexes, and decreased ATP generation in neurogenesis, synaptogenesis, and synaptic plasticity during brain development.

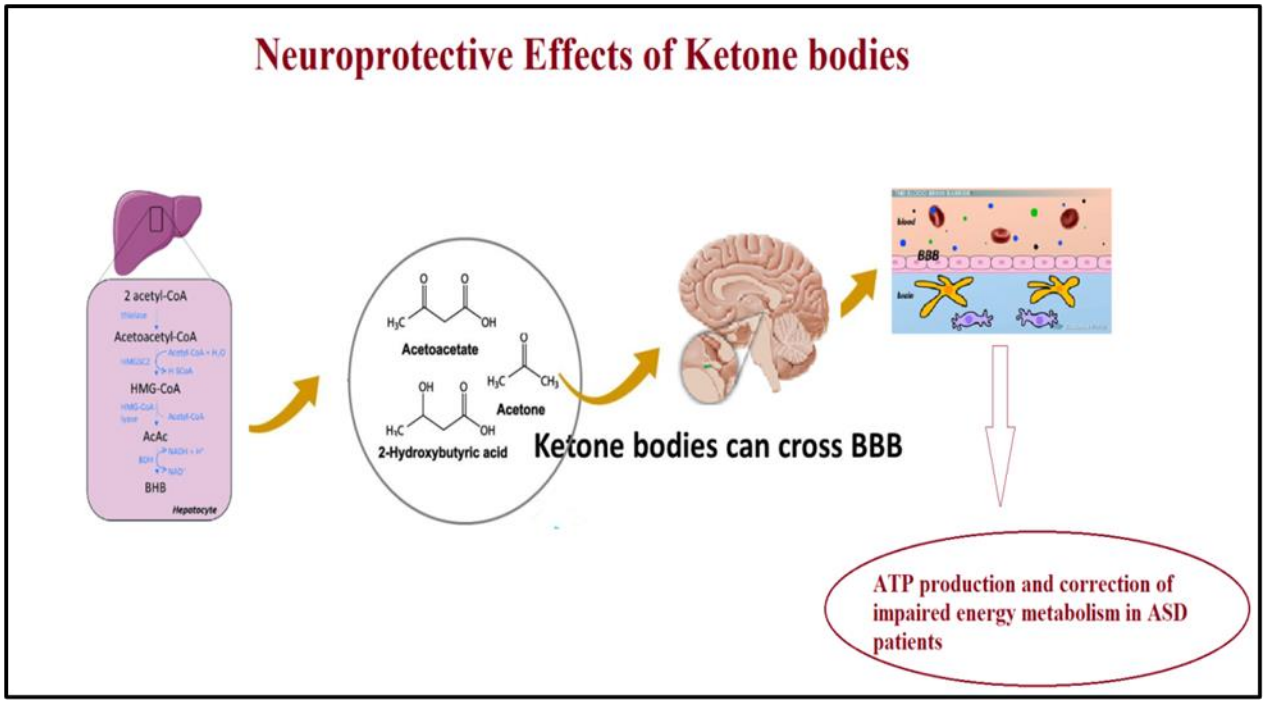

Figure 5: Neuroprotective effects of ketone bodies that cross the blood brain barrier to nourish neurons and produce ATP during low-carbohydrate diets.

These molecules also help to prevent transitions in mitochondrial permeability and reduce reactive oxygen species (ROS) (Kim et al., 2007& 2015). Thus, ketone bodies have neuroprotective effects on the central nervous system (CNS). A KD is considerably effecient in treating epilepsy as commonly known comorbidity in ASD. A 56% reduction in mean seizure frequency was recorded in refractory epileptic patients 1–18-years-old- treated with a KD for 4 months (Lambrechts et al., 2017). A KD may also mend some core symptoms of ASD and comorbidities, but records from clinical trials on KDs as a treatment for ASD are very limited. The present review examined the role of a KD in ASD treatment and discussed the underlying mechanisms. The neuroprotective effects of KBs are attributed to the fact that most neurons do not efficiently produce ATP from fatty acids, but KBs can be a source of ATP when carbohydrates are less available (Cullingford et al., 2002, Trimboli et al., 2020).

Multiple studies have focused on change in the gut microbiota as a risk factor in individuals who are genetically predisposed to ASD; these alterations in the gut microbiota may influence the risk of ASD by modifying the immune system and metabolism (Coury et al., 2012; De Angelis et al., 2015). Breastfeeding is associated with a lower risk of ASD if continued for 6 months, while formula-fed infants show a higher amount of Clostridium difficile in the gut (Schultz et al., 2016; Azad et al., 2013). Furthermore, C. difficile has been hypothesized to be a potential risk factor for ASD, supported by a study in which children with regressive autism were treated with a 6-week oral course of vancomycin (an antibiotic used against C. difficile), which resulted in a significant improvement of both neurobehavioral and gastrointestinal symptoms (Sandler et al., 2000). However, relapsing gastrointestinal and neurobehavioral symptoms occurred gradually after treatment interruption, possibly because the spores of C. difficile were

resistant to vancomycin and could turn into infective forms later (Ding et al., 2017, Yang et al., 2018).

From the precision medicine perspective, the use of probiotics as a treatment strategy for ASD be tested, taking into account the presence/absence of GI problems. Santocchi et al. (2020) suggested that ASD children with and without comorbid GI problems could represent two different groups and that probiotic interventions could potentially result in different effects, likely due to the distinctive targeted microbiota. Previous studies have already suggested that differences in gut microbiota are independent of GI dysfunction. Luna et al. (2016) suggested that larger and well-designed studies are still needed before it can be concluded whether microbial composition may stratify ASD children without GI problems. A positive impact of probiotics on autism severity in children without GI problems supports the contribution of the microbiota-gut-brain axis as an etiological mechanism of this ASD subgroup (Luna et al., 2016; Arnold et al., 2019).

In the subgroup of ASD children with GI dysfunction, a positive effect of probiotics was recorded not only on GI symptoms but also on core symptoms of ASD, including adaptive functioning, developmental signaling, and multisensory processing (Santocchi et al., 2020). This novel finding of a significant improvement in multisensory processing in the GI group could reveal the complex interaction between these two classes of symptoms and their effects on development and adaptive functioning.

Specifically, probiotic supplementation, acting on dysbiosis, could relieve pain and enteroception caused by GI problems and, accordingly, could improve the multisensorial integration process, which in turn is affected by averse enteroceptive stimuli in dysbiotic patients. Moreover, dysbiosis could impact neurotransmitters that play a role in sensory developmental pathways. Recently, difficulties in multisensory processing have been related to serotoninergic neurotransmission (Siemann et al., 2017), and serotonin levels are controlled by the gut microbiota. Thus, we hypothesize that probiotics could ameliorate sensory abnormalities by restoring the serotoninergic system, which also works to reduce GI dysfunction comorbidities.

Based on the above findings, the therapeutic effects of probiotic supplementation on the NGI and GI groups of autistic children may be due to different mechanisms. Thus, each medication is likely to benefit only a subgroup of patients within the spectrum, which could be used to ascertain the importance of biomarkers for personalized precision medicine (Thye et al., 2017, Hollander and Uzunova, 2017). Understanding the mechanism behind the positive effect of probiotic treatment on both GI and NGI children would greatly facilitate the identification of ASD subjects who can respond to probiotic supplementation regardless of the presence of comorbid GI dysfunction and GI inflammatory grade (Veenstra-VanderWeele et al., 2017).

To our knowledge, selection of the proper probiotic could be based on the presence or absence of imbalanced GABA/Glu, hyperserotonemia, and depletion of neuropeptides or metabolites (all ASD biomarkers). It is well known that germ-free (GF) animals have much lower brain levels of glutamine (involved in the production of GABA and Glu), tyrosine (a precursor of dopamine and noradrenaline), and tryptophan (a precursor of 5-HT). For example, in the case of ASD patients with glutamate excitotoxicity, administration of Lactobacillus rhamnosus is recommended, as it induces GABAA and GABAB receptors in specific brain regions, accompanied by declines in anxiety and depression-associated behaviors. Janik et al. (2016) used magnetic resonance spectroscopy (MRS) to demonstrate marked increases in glutamate+glutamine (Glx) and total N-acetyl aspartate+N-acetyl aspartyl glutamic acid (tNAA), as well as GABA, the primary inhibitory neurotransmitter, in L. rhamnosus-treated patients. Collectively, these results suggest that L. rhamnosus is a psychoactive bacterium that is effective in correcting the etiological mechanism of E/I imbalance in ASD. Based on these results, the application of standard clinical neurodiagnostic practices (e.g., MRI), translational opportunities (probiotic supplementation), and regular assessment of related biomarkers (Glx, tNAA, GABA, and GABA receptors) could greatly benefit the field of personalized precision medicine. Interestingly, bacterial Glu is a substrate for GABA synthesis through decarboxylation by Glu decarboxylase (GAD), and has been detected in both gram-positive and gram-negative bacteria such as Lactobacillus spp., Lactococcus spp., and Streptococcus spp. (Cotter and Hill, 2003; Nomura et al., 1999).

Hyperserotonemia may also be a biomarker of ASD. Some treatments involving microbial management have potential, including those involving probiotics or fecal transplant. Mice administered Lactobacillus reuteri demonstrate improvements in the GI tract and behavioral alterations (Kahneman et al., 2009; Karlan and Appel, 2011). Trials evaluating the microbiome, 5-HT, and gastrointestinal dysfunction in ASD (Hotz et al., 2005). Findings in humans, however, have been limited, and large prospective trials are needed.

Alterations in intestinal barrier integrity have been recognized in rodent models and patients with ASD (de Magistris et al., 2010). Fiorentino et al. (2016) reported reduced expression of tight junction components in 75% of patients with ASD. Zonulin controls tight junctions between enterocytes and is a physiological modulator and excellent biomarker of intestinal permeability. Zonulin is increased in patients with ASD compared with healthy controls and is correlated with ASD severity as measured by the Childhood Autism Rating Scale (CARS) (Esnafoglu et al., 2017). Zonulin may be a promising biomarker for role of other probiotics proved that a probiotic mixture of Lactobacilli and Bifidobacteria, particularly L. acidophilus, L. plantarum, L. helveticus, L. paracasei, B. breve and B. lactis, can improve GI symptoms and quality of life in ASD patients (Kasy, 2016) and identified significant associations between the

the subgroup of children with ASD and GI problems stemming from altered intestinal integrity. However, it should also be noted that not all studies support that theory of increased gut permeability (Esnafoglu et al., 2017).

Interestingly, the identification of biomarkers of ASD risk would be particularly useful in estimating whether a child will develop an ASD before it clinically presents and to provide sufficiently early intervention during development. Validation of predictive biomarkers requires determining their accuracy (i.e., sensitivity, specificity, positive and negative predictive values), plausibility (causal or mechanistically understandable), reliability in relating to a certain clinical endpoint, and reproducibility across clinically relevant settings (Prata et al., 2014).

Biomarker	Body sample	Variation		ROC analysis			P value	Reference
		Higher	Lower	specificity	Sensitivity	AUC	0.001	Alabdali et al., 2014
Neurotransmitters markers								
GABA	Plasma platelet free	*		90%	80%	0.883	0.001	
Serotonin			*	100.0%	100.0%	1.000	0.001	
Dopamin			*	92.9%	88.9%	0.968	0.001	Zhang et al., 2014
Oxytocin			*	90%	100.0%	0.981	0.001	
Brain derived neurotrophic factor (BDNF)	Serum			71.7%	86.7%	0.83	0.001	
glutamate	Plasma		*	----	----	----	0.0001	Talitha et al., 2017
glutamate	Brain using	*					= 0.006	
GABA			*					
Glutamate/GABA			*				0.003	

	(1H-MRS							
Oxidative stress markers								
Total glutathione	Plasma		*	100%	20%	1.000	0.001	Al-Yafee, et al., 2011
GSH/GSSG			*	100%	20%	1.000	0.001	
Peroxiredoxin 1		*		90%	80%	0.915	0.001	
Peroxiredoxin 3		*		100%	85%	1.000	0.001	
Thioredoxin 1		*		100%	85%	1.000	0.001	
Thioredoxin reductase		*		65%	85%	0.881	0.001	
Isoprostane		*		100%	78%	1.000	0.001	El-Ansary and L. Al-Ayadhi., 2012a
Neuroinflammation markers								
IL-6 combined with Serotonin		*		87.88%	74.19%	0.85	0.001	Yang et al., 2015
IL-6		*		84.85%	96.77%	0.96	0.001	
HSP-70		*		95.0%	84.2	0.987	0.001	El-Ansary and L. Al-Ayadhi., 2012b
TGF-β		*		100%	89.5%	1.000	0.001	
Caspase-7		*	*	90.0%	89.5%	0.968	0.001	
Caspase -3				100%	86.7%	0.968	0.001	El-Ansary et al., 2011a
Neopterin		*		84.2%	80.1%	0.876	0.0001	Zhao et al., 2015
Lipoxin A4			*					
Toxic biomarkers								
Urinary phthalate	Urine	*		52.1%	75.6%	0.638	<0.05	Testa et al., 2012
Zinc/copper			*	90.0%	91.7%	0.968	0.001	
lead	Serum	*		Satisfactory specificity and sensitivity			0.001	El-Ansary et al., 2011b

Mercury	brain, blood, urine, teeth, hair, and nails	*						Kern et al., 2016
Aluminium	Brain	*						Skalny et al., 2017
Mn, Al, Cd, Cr, Cu,	Hair and serum	*						Tinkov et al., 2019
Pb, Cd, As, Cu, Zn, Fe, Hg, Ca, Mg	Hair	*						Zhai et al., 2019
Vitamins								
Vitamin D	serum		*				0.006	Petruzzelli et al., 2020
Vitamin A	serum		*				0.001	Wen et al., 2021
B6, B9 and B12	Urine		*				0.05	Belardo et al., 2019
B12 and Folic acid	Serum		*				0.001	Mahruba et al., 2019
Vitamin E	Serum		*					Fido and Al-Saad, 2005

Table 1 List of the most common predictive biomarkers in ASD, sensitivity, and specificity as calculated by the Reciever Characterestic Operative (ROC) analysis.

5. Concluding Remarks

The recent hypothesis that multiple etiological and pathophysiological mechanisms are involved in ASD makes personalized precision medicine the most recommended approach for successful treatment and intervention strategies for individuals with ASD. Additionally, a complete understanding of an individual's cognitive, social, and sensory profiles and the relationship between the treatment outcome measures of these profiles (CARS, SRS, and sensory profile) and the severity of the clinical presentation in ASD could greatly benefit patients with ASD; their families may be more likely to accept future precision medicine trials if these trials were able to accurately provide prognoses for their ASD children.

In ASD, interventions are most efficient and successful if they commence as early as possible; however, diagnosis often occurs late, partly because the diagnosis of ASD is primarily based on recognizing autistic behaviors that may not appear until the disorder is already clinically presented. Retrospective and prospective studies dealing with clinical features and diagnostic criteria of ASD have examined the behaviors of ASD children usually obtained through their parents, screening tools, and videos (Costanzo et al., 2015). The most common

early signs involve eye contact, mutual responsiveness, turning to verbal call, facial communication, and social interaction. Different data are described about the age at onset of the early signs: for instance, the first noticeable social interaction impairment may occur between 2 and 6 months (Jones and Klin, 2013); sleep quality, feeding and temper problems may occur during the first year in children at risk for ASD (Garon et al., 2009; Richdale and Kimberly, 2009; Emond et al., 2010). The beginning of features is considered by a worsening language and social interaction nearby 16–20 months [Ozonoff et al., 2010], sometimes by a psychomotor delay (Siperstein et al., 2004).Based on this delay, identifying biomarkers that indicate at-risk children during the presymptomatic period could greatly facilitate early diagnosis, confirm behavioral abnormalities, classify patients into subgroups, and predict personalized precision treatment strategies related to the biomarkers in different subgroups.

Recently, unsuccessful clinical trials were attributed to theoretical and methodological concerns related to the translatability of research data from animal models to humans, the design of different clinical trials, placebo effects, and objective measures of clinical trial outcomes. In the future, translatable progressive multidisciplinary joint integrated interventions are required to overcome these concerns.

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