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AN EXTENSIVE REVIEW ON EFFECTS OF PSYCHOBIOTIC BASED TREATMENTS IN AUTISTIC SPECTRUM OF DISORDER AND APPLICABILITY OF ZEBRAFISH AS A ROBUST MODEL FOR STUDYING ASD

Anusha Samanta and Tamalika Chakraborty *

Department of Life Science, Guru Nanak Institute of Pharmaceutical Science and Technology, Kolkata - 700114, West Bengal, India.

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Correspondence to Author:
Ms Tamalika Chakraborty

Assistant Professor,
Department of Life Science,
Guru Nanak Institute of Pharmaceutical Science and Technology, Kolkata - 700114, West Bengal, India.

E-mail: tamalika.chakraborty@gnipst.ac.in

ABSTRACT: Autism spectrum disorders (ASDs) refer to the group of neuropsychiatric disorders that alter neurodevelopment and mental function and are characterised by an extensive range of symptoms, including hyper-or hyposensitivity, repetitive behaviours, and impairments in social and academic functioning. Untangling the causation of ASD has been complicated, in part because distinct genetic abnormalities can result in both identical and distinctive traits that entirely fall within ASD. ASD can be triggered by hereditary and/or environmental causes. Studies in both experimental and clinical trials have revealed that the gut microbiome is altered in ASD patients. Due to its linkages to nutrition and the reciprocal relationship with the host, these changes affect the composition of the gut microbiota and also the metabolites they create. The gut-brain axis (GBA) mediates how the central nervous system (CNS) associated roles and behaviours are affected by psychobiotics, a unique family of probiotics that enhances not only gastrointestinal (GI) function but alsoanxiolytic and antidepressant activity. Psychobiotic use can enhance GI processes and ASD-related symptoms. New models for studying ASD have been developed during the past few decades, *in-vitro* also *in-vivo*. These models hold great promise for validating few of the formerly identified risk factors for the disorder's onset and for testing novel possible treatments, like the use of psychobiotics to minimise ASD symptoms. The evidence supporting psychobiotics' impact on ASDs is still limited, nevertheless. Future research on the efficiency and mechanism of psychobiotics as treatments for ASDs will be necessary using more effective models *e.g.*, zebrafish.

INTRODUCTION: A range of diverse neurodevelopmental disorders (NDDs) known as autism spectrum disorders (ASDs) are influenced by both hereditary and environmental factors [1]. Individuals with the presence of social and communication deficits as well as a lack of universal interpersonal skills are considered of having ASDs.

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Patients may also experience repetitive or stereotyped forms of behaviour, interests, and/or actions in addition to these symptoms. The 5th edition of "Diagnostic and Statistical Manual of Mental Disorders" sets forth criteria for the diagnosis of ASDs.

According to that, the NDDs listed below fall under the ASD category: Asperger's disorder, Kanner's autism, pervasive developmental disorder not otherwise specified (PDD-NOS), high functioning autism, atypical autism, early infantile autism, and childhood disintegrative disorder (American Psychiatric Association 2013) [2]. ASD is, globally, a significant contributor to developmental disability. The "World Health Organization" (WHO) defines

the incidence of ASD globally at 0.76% but only accounts for roughly 16% of all children worldwide [3]. Its estimated prevalence in the UK is 1% [4], and in the US, parent-reported ASD diagnoses in 2016 had an average of 2.5% [5]. There are around 1.3 billion people living in India, with children below the age of 15 having to make up about one-third of the population. ASD may affect more than 2 million individuals in India, according to estimates. The majority of published research on ASD relies on hospital-based data, hence they lack data on the disorder's estimated prevalence in India. Only a few research have specifically examined its prevalence in community settings. Furthermore, it is challenging to determine the exact occurrence of ASD due to the inconsistent use of properly validated and translated diagnostic methods for autism. Because of a suspension in ASD diagnosis at a young age, the illness is also not fully recognised [6].

The development of prospective treatments has been delayed by the difficulty in determining the pleiotropic character and genetic complexity character of ASDs, as well as a variety of putative environmental causes [7, 8]. Most prominently, counting Prader-Willi and Angelman syndromes, 1-3% of all cases of autism are associated with duplications of the 15q11-q13 region that are inherited from the mother [9]. 4–5% of ASD cases had mutations at established ASD sites, like those in Rett and Fragile X syndrome [10].

Despite being highly heritable illnesses, ASDs are only heritable in about 50% of cases, and even monozygotic twins do not always have identical twins [11]. Recent transcriptome investigations have shown that immunological maturation and neural development are both linked to ASD, and enrichment analysis has identified cellular and molecular pathways e.g., synaptic function and WNT signalling [12]. Many genes which regulate the synapse also are revealed primarily in the elated inhibitory neural lineages were discovered in a current whole-exome sequencing analysis of ASDs [13]. Another recent study links ASD in mice and humans with insufficient myelination brought on by oligodendrocyte dysregulation [14]. Antibiotic use, epigenetic alterations, and the maternal and baby microbiota have entirely been linked to the growth and advancement of ASD pathogenesis.

Recent studies have exposed that ASD sufferers are known to experience digestive issues and different microbiome taxonomic patterns from neurotypical (NT) people [15]. The “gut-brain axis” is thought to be a two-way transmission channel involving the brain and the gut. Still, this idea might be broadened to make the community of microorganisms a crucial component of this triangle [16]. Antibiotic use influenced the enteric nervous system (ENS) also the central nervous system (CNS), and exposure to infections altered gut-brain symptoms as well as immune activation. In the opposite circumstance, microbiota-targeting antibiotics have been used to successfully treat hepatic encephalopathy [17]. The neuroendocrine, neuroimmune, and autonomic nervous systems all perform a role in the connection or transmission between the microbiota in the stomach also the brain [18].

Around 9 to 84% of people with ASD report having GI issues as a comorbidity[19]. These include diarrhoea (19%) and constipation (20%), both of which are more common in children having ASD than in their healthy brothers or sisters (42 vs. 23%, correspondingly) [20]. The data connecting the gut microbiota either directly or indirectly to the symptoms of ASD suggests that this may occur in part due to its impact on the host metabolism also its defence mechanism [21, 22]. One of the diseases associated with ASD patients is leaky gut or increased intestinal epithelial permeability [23].

Compared to the control batch (4.8%), patients with ASD (36.7%) also their family members (21.2%) demonstrated greater percentages of aberrant intestinal epithelial permeability [24]. Increased permeability makes it possible for toxins also bacterial by-products to go into circulation, which ultimately affects brain function with lowers social behavioural scores [18]. Recent research indicates strong links between the gut microbiota also the aetiology of ASD, with bacterially obtained metabolites from the GI tract linked to changes in host neurodevelopment along with neural-specific mRNA processing, even though our understanding of the relationship between the microbiome and ASD is still limited [25, 26].

Probiotics Affecting Gut-Brain Axis – Psychobiotics in Mental Health: The sum of

bacteria in the human digestive tract, 10^{14}, stands 100 times above the total number of cells in the body. Numerous physiological systems, such as energy balance, ENS activation, and immunomodulation, have been demonstrated to be influenced by gut bacteria or an individual's microbiota profile influenced by d Diet, genetics, sex, age, and other variables [27]. The relationship among gut dysbiosis along with functional gutdisorders and CNS disorders provides implications for the microbiota-gut-brain axis (MGBA) connection [28, 29]. The idea compared to specific pathogen-free (SPF) controls of MGBA was established by the reduced social behaviours of germ-free (GF) animals. Another indication of the significance of gut flora to CNS functioning is the improvement of social impairments in GF mice following the transplantation of a typical microbiome [30].

A unique class of probiotics known as "psychobiotics" suggests potential uses in the therapeutics of psychiatric disorders by regulating gamma-aminobutyric acid (GABA), brain-derived neurotrophic factor(BDNF),glutamate, and serotonin, that perform crucial parts in managing the neuronal excitatory-inhibitory stability, cognitive abilities, mood, learning as well as memory processes [31, 32, 33]. Immunoregulatory, neuroendocrine, and vagus pathways have all been proposed as potential channels of transmission among the brain and gut microbiota [34]. Some Lactobacillus and Bifidobacterium strains, including *Lactobacillus plantarum*, *Bifidobacterium dentium*, and *Lactobacillus brevis* create serotonin and GABA, while other Lactobacillus strains, including *L. plantarum* and *L. odontolyticus*, produce acetylcholine [35, 36, 37, 38]. It has recently been discovered that bacteria can control the synthesis of serotonin in the gut as enterochromaffin cells in the stomach stimulate serotonin production when exposed to spore-forming bacteria from the gut microbiota [39]. Erythrogenic cytokines can be decreased by probiotics e.g., *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* [40]. Probiotics have been revealed to have anti-immunoregulatory effects that activate the T regulatory cell population and IL-10 production [41].

Additionally, probiotics interact with GI epithelial enteroendocrine cells (EECs) for the formation of neuropeptides also neurotransmitters like substance P, neuropeptide Y (NPY), serotonin, peptide YY (PYY), glucagon-like peptide-1 and -2 (GLP-1 and GLP-2) and cholecystokinin [42, 43]. ENS neurons and gut EECs are the primary sources of serotonin, which is associated with the control of GI secretion and motility [44]. In **Table 1**, some pieces of evidence of psychobiotics having psychotropic reactions to stress, anxiety and also depression has been shown. Regarding the potential uses of psychobiotics, the investigations have yielded some encouraging data, however, there is still unavailability of human data. In this area, additional clinical research is indispensable.

TABLE 1: EXAMPLES OF SOME RECENTLY OBSERVED PSYCHOTROPIC EFFECTS OF SOME PSYCHOBIOTIC STRAINS IN STUDY MODELS

Strain	Effects on study models	Reference
Lactobacillus plantarum PS128 (PS128)	Corticosterone levels and behaviours resembling depression and anxiety were reduced. Dopamine and serotonin levels in the striatum and prefrontal cortex are elevated.	45,46
Lactobacillus helveticus NS8	The levels of serotonin, norepinephrine (NE), and brain-derived neurotrophic factor (BDNF) in the hippocampus are enhanced. Anxiety, depression, and cognitive impairment are decreased.	47
Bifidobacteriu longum 1714	The symptoms of stress, despair, and anxiety are reduced.	48
Lactobacillus rhamnosus (JB-1)	Depression and anxiety are reduced. The amount of plasma corticosterone (CORT) is decreased causing region-dependent changes in the brain's GABA receptor expression.	49
Bifidobacterium longum NCC3001	Anxiety is treated. BDNF expression in the hippocampus is uplifted.	50
Bacterium infantis 35624	Depression-like behaviours are healed.	51
Bifidobacterium longum 1714	Stress is decreased. Memory is improved.	52
Probiotic yogurt: *Bifidobacterium lactis BB12 Lactobacillus acidophilus LA5* Probiotic capsules: *Lactobacillus*	Mental health characteristics are boosted by evaluating the depression anxiety and stress scale (DASS) also general-health questionnaire (GHQ).	53

acidophilus, L.casei, L. bulgaricus, L. rhamnosus, Streptococcus thermophiles, Bifidobacterium longum, B. breve L. helveticus R0052 *Bifidobacterium longum* R0175	Anxiety, depression, and the level of urine-free cortisol are decreased.	[54,55]

Modelling for ASD: Patients with ASD typically receive treatment aimed at easing the disorder's symptoms to lessen how much of an impact they have on their daily lives. To do this, it is common for patients to receive a mix of therapeutic modalities, such as behavioural therapy and/or medication. There is no drug that can totally cure ASD or lessen its symptoms. Moreover, it has been mentioned previously that risk factors for developing ASD include both hereditary and environmental elements. Hence, it was very challenging for the researchers to find out the individual effects of each risk factor in the occurance of ASD. For that, the development of several in vivo and in vitro pre-clinical models of human illnesses that could assist in understanding the molecular processes that underlie the emergence of a particular pathology has been observed [56].

Genome Editing System: A promising Tool for modelling human disorders is genome editing Systems. The current three foremost types of genome-editing technologies are Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and CRISPR/C as (Clustered Regularly Interspaced Short Palindromic Repeats). Each of these three systems causes distinct DNA breaks that set off the cellular DNA repair processes. The most recent genomic editing technology, CRISPR/Cas9, is rooted in the bacterial defence mechanism CRISPR type II and differs from ZFN and TALENs in that it only requires two components to operate: Cas9 nuclease and single guide RNA (sg RNA) [57].

Furthermore, several enzymes can replace Cas9, increasing the technique's usefulness. In order to increase the variety of CRISPR/Cas modifying enzymes available to us, it may be helpful to develop new types of Cas nucleases, but it's also intriguing to consider whether existing nucleases like Cas9 could be modified. For instance, much work has gone into creating Cas9 that is induced, also modifying its recognition site (PAM sequence), and increasing its exactness [58, 59]. The functional basis for CRISPR interference (CRISPRi) [60], CRISPR activation (CRISPRa) [61], base editing [62], and prime editing [63] is a modified Cas9 that can be coupled with various enzyme domains. The existence of inaccurate impact in the genome of the modified cells is a significant downside of CRISPR/Cas technology, which can be particularly risky for therapeutic applications [64]. Several model organisms, including zebrafish [65], Drosophila melanogaster [66], rodents [67], *etc*., have been successfully edited genetically using the CRISPR/Cas system. Human somatic [57] and embryonic cell lines [68] have both been cultured using CRISPR/Cas.

***In-vitro* Modelling of ASD:** It was not possible to use cell culture as a model for ASD because there was little chance of collecting CNS cells through biopsies. This obstacle has been overcome in 2006 at the time when Yamanaka and his colleagues discovered pathways that permit reprogramming grownup somatic cells by enabling the conversion of altered cell lines to induced pluripotent stem cells (hiPSCs) by introducing four genes i.e., the Yamanaka factors (Oct3/4, Sox2, Klf4,and c-Myc). Now it is feasible to create cell lines precisely from patients thanks to the development of modern reprogramming techniques and differentiation regimens. As a result, it is possible to create specific in vitro models to investigate the underlying causes of a certain condition in an individual. Patient-derived cellular models have been verified to be extremely strong, trustworthy, and realistic while preserving the genetic makeup of the origin.

Since, they complement the genetic makeup of the patients, it is possible to examine the biological basis of each condition. These models are helpful for determining the connection between a genotype and phenotype as well as for creating new therapeutic strategies, such as cell therapy and pharmaceutical remedies. Drug sensitivity tests, which are useful to authenticate the activity of the chosen medications prior to medical assays, can help achieve this by helping to identify new targets

for therapy or biomarkers. This method gives up fresh opportunities for the investigation of the molecular origins of convoluted illnesses like ASD for all the previously mentioned reasons [68, 69, 70, 71]. Even with the benefits of *in-vitro* models, which will never fully replicate the complexities behind their development, animal models remain an essential tool for understanding ASDs.

Animal Models for ASD: The use of animal models in ASD research has the potential to be significant. For the research of autistic disorders, there are numerous genetic models available [72]. Animal models do not, however, display all the signs of human neurodevelopmental disorder. Animal models for ASD have been found using two basic methods. Forward genetics is the first method, in which ASD-like traits are found in the chosen animal model, after which the molecular causes of the detected changes are clarified. Reverse genetics is the second method, in which certain mutations are inserted into the animal model's genome after which the phenotype is identified [73]. Typically, laboratory-bred rodents like rats or mice have been mostly used in ASD research experiments [74]. Rodents are ideal for the research of ASD as their social behaviour has been extensively examined by a set of tests like the three-chambered social interaction, the Morris water task, swimming tests, or even just a simple assessment of exploratory behaviours, and their neurological system may be manipulated using a variety of well-established methods. Furthermore, since rats and mice are sociable, it is well-known how they relate to one another in terms of parental, sexual, and territorial activities. Rats were used in the first ASD investigations because of their obvious social behaviour. However, because mice are less expensive, their use in ASD research has been rising [75].

***Mus Musculus* or house mice in ASD Research:** Despite the abundance of ASD genetic models, there are fewer and largely inbred (*e.g.*, Black and Tan Brachyury, or BTBR) or environmental (*e.g.*, VPA, MIA) animal models available for the research of the gut microbiota-ASD connection [72]. The BTBR mouse strain has been utilised in numerous investigations evaluating the impact of medications and gut microbiota products on ASD-related outcomes because it consistently replicates the ASD phenotype in various lab settings[76]. The C57Bl/6J mouse strain, which is more motivated and less impulsive, is another one used in ASD research [77].

As a maternal immune activation (MIA) mouse model, pregnant C57Bl/6J females were also administered *B. fragilis*. Due to the disruption of the GI barrier, which results in inflammatory actions that may eventually influence the neuro-development of the progenies, MIA, throughout pregnancy, has been proven to upsurge the risk of developing neurodevelopmental psychiatric problems like ASD [78, 79].

***Rattus norvegicus* or Common Rats in ASD Research:** Rats have been suggested as a model creatures with a great possibility to study NDDs, counting ASD, due to their more complicated behaviour and social interactions. With the use of ZFN and also ENU-induced mutagenesis, the first rat knockout models for the investigation of ASD were produced in 2010 [80]. However, despite the clear applicability of rodent models for ASD modelling along with the priceless data they provide, there are yet some glaring limitations that have prompted scientists to choose more controllable models.

Clinical Studies of ASD: Several recent clinical trials of psychobiotics mentioned in the U. S. National Library of Medicine have been listed down below **Table 2**. Some of these trials are either completed successfully or still in the process of examining.

TABLE 2: RECENT CLINICAL TRIALS OF PSYCHOBIOTICS IN ASD-RELATED HUMAN SUBJECTS MENTIONED IN THE U.S. NATIONAL LIBRARY OF MEDICINE [27]

Intervention/ treatment	Subjects	Time of intervention	Primary outcomes	Secondary outcomes
Dietary Supplement: Synbiotic (probiotic + prebiotic) *Bifidobacterium infantis* SC268 bovine colostrum, bovine oligosaccharide Dietary	2 Years to 11 Years (Child) (all sex)	5 weeks	Changes in Stool Microbiota Composition Change	Serum Immune Profile Change

Supplement: Prebiotic bovine colostrum, bovine oligosaccharides				
Oral symbiotic [*Lactobacillus rhamnosus* (1x1010 CFU/dose), *Lactobacillus plantarum* (4 x 109 CFU/dose), *Bifidobacterium animalis* subsp. lactis (5 x 109 CFU/dose), *Bifidobacterium longum* (1 x 109 CFU/dose) + 4g/dose of partially hydrolysed guar gum (PHGG)] + gut-directed hypnotherapy	5 Years to 10 Years (Child) (all sex)	Baseline12 weeks24 weeks	Changes in GI symptom severity by 6-GSI (6-item gastrointestinal severity index)	Changes in ASD severity/behaviour by ABC (Aberrant Behaviour Checklist) questionnaire, Anxiety by PRAS-ASD (Parent Rated Anxiety Scale - Autism Spectrum Disorder) questionnaire, Gut microbiome by stool analysis
PS128: *Lactobacillus plantarum* PS128 (>3 × 1010 CFU) Placebo: microcrystalline cellulose	Baseline 12 weeks 16 weeks	7 Years to 12 Years (Child) (both sex)	Changes in total scores on the Social Responsiveness Scale	Changes in total scores of Repetitive Behaviour Scale-Revised, Child Behaviour Checklist, Adaptive Behaviour Assessment System, Aberrant Behaviour Checklist, Emotional Dysregulation Inventory, Parenting Stress Index Change in the accuracy of Frith-Happe animation, Eyes task Visual Analogue Scale for GI Symptoms
Lactobacillus reuteri DSM 17938 + *Lactobacillus reuteri* ATCC PTA 6475+ placebo	3 months 6 months	18 Months to 8 Years (Child) (both sex)	Changes in severity level of ASD symptomatology and Microbiome Profile	Changes in GI symptoms, ASD symptomatology: problematic behaviours and repetitive behaviours, Adaptive Functioning, Parental Stress, Metabolomic Profile, Inflammatory Profile
Oral symbiotic: Prebiotics (human milk oligosaccharides/HMOs) + probiotics (*Lactobacillus rhamnosus, Lactobacillus plantarum, Bifidobacterium animalis spp*. lactis, and *Bifidobacterium longum*- 20 billion CFU) Placebo: powdered maltodextrin	8 weeks 17 weeks	5 Years to 12 Years (Child) (both sex)	Behaviour change	Change in GI Symptom Severity, Gut Microbiome, Anxiety Levels, Quality of Life, Stool Consistency, stool short chain fatty acids levels, urinary serotonin concentration, salivary cortisol levels
Vivomixx®: *Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Placebo: maltose and silicon dioxide	3 months 6 months	18 Months to 72 Months (Child) (both sex)	Changes in severity level of ASD symptomatology	Changes in GI symptomatology, Electroencephalogram (EEG) power, levels of serum like Lipopolysaccharide, Leptin, Resistin, Tumor Necrosis Factor - alfa, Interleukin-6 (IL-6), Plasminogen Activator Inhibitor-1 (PAI-1), levels of fecal calprotectin, ASD symptomatology, Adaptive Functioning, Behavioural Profiles, Parental Stress
Vivomixx® + Placebo (maltose and silicon dioxide)	4 weeks 12 weeks	3 Years to 16 Years (Child) (both sex)	Change in the Autism Treatment Evaluation Checklist(ATEC) Total Score	Change in the Frequency of GI Symptoms, the ABC Section Scores, the Autism Parenting Stress Index (APSI) Score
Visbiome: *L. acidophilus*	8 weeks 3	Years to 12	Change in GI Module	Change in Target Symptom

DSM24735, *L. plantarum* DSM24730, *Lactobacillus paracasei* DSM24733, *L. helveticus* DSM24734, *Streptococcus thermophilus* DSM24731, *B. lactis* DSM24736, *B. breve* DSM24732, and *B. lactis* DSM24737. Placebo: Maltose with silicon dioxide		Years (Child) (both sex)	of the Pediatric Quality of Life Inventory (PedsQL)	Rating, Parent Anxiety Checklist—ASD, The ABC, Social Responsiveness Scale (SRS), Children's Sleep Habits Questionnaire (CSHQ), The Parenting Stress Index (PSI)
B. lactis BB-12+ *L. rhamnosus* GG (BB-12+LGG) Low dose: 1 billion CFUs High dose 10 billion CFUs Placebo: Maltodextrin	84 days	4 Years to 16 Years (Child) (both sex)	Adverse events (Safety) reporting	Changes in irritability and maladaptive behaviours with ABC and the Social Responsiveness Scale-2
GRAS approved probiotic strains, prebiotics, and botanicals	3 months	30 Months to 75 Years (Child, Adult, Older Adult) (both sex)	Changes in Parent Global Impressions of Autism	Changes in SRS, GI Symptom Rating Scale
Lactobacillus plantarum PS128 6×1010 CFU. Placebo: Microcrystalline cellulose	Baseline 8 weeks 12 weeks 20 weeks	2 Years to 5 Years (Child) (both sex)	Change in Autism Diagnostic Observation Scale (ADOS), SRS, Social Communication Questionnaire (SCQ), ABC, Childhood Autism Rating Scale (CARS), Gesell developmental scale, frequency of child behavioural problems and ASD symptoms, GI symptoms among children with ASD	Cytokine Analysis Evaluate changes in type, number, and structural composition of gut microorganisms in children with ASD
Oral probiotics + intranasal oxytocin	Baseline 16 weeks 28 weeks	3 Years to 25 Years (Child, Adult) (both sex)	Changes in SRS, ABC	Changes in Neuroinflammation and Oxytocin levels, structural and functional MRI, Autonomic indices, Microbiome, Eye tracking, and Behavioural task
Oral probiotics/; *Lactobacillus rhamnosus* - ATCC 21052, *Lactobacillus plantarum* - ATCC 8014 and *Bifidobacterium longum* subsp. Infantis-ATCC 15707 (one billion CFU/g) Placebo	24 weeks	2 Years to 18 Years (Child, Adult) (both sex)	Change in childhood autism rating scale	----------

Zebrafish Model: Reasons to accept it to Study ASD: Zebrafish have been proposed as asupreme animal model in recent years for the investigation of the genetic basis of a number of human diseases also astonishingly, they are currently used as a model in more than 800 laboratories worldwide [81]. Over 70% of the zebrafish genome is similar to human genes, and zebrafish and human neurodevelopmental processes are the same [82]. Compared to more conventional model systems, this one has certain clear advantages [73]. For instance, zebrafish embryos are transparent and develop quickly in the external environment, making it possible to directly see neurodevelopmental processes and brain activity in a healthy, functional nervous system. Additionally, zebrafish are very tractable and have substantial offspring, making it possible to conduct high-throughput pharmacological screens on a larger scale than is possible with rodent models.

Furthermore, with improvements in zebrafish CRISPR/Cas9 gene-editing methods, it is now possible to quickly and cheaply produce mutant zebrafish with loss-of-function mutations in a gene of interest [83, 84]. Zebrafish, therefore, have great promise for enhancing our knowledge of the functions of risk genes inneurodevelopment and illuminating the fundamental biological processes behind NDDs.

Zebrafish are effective model organisms for studies examining the role of the microbiome. It is conceivable to nurture them in sterile environments that produce germ-free individuals because they are initially colonised by microorganisms through their environment. Experiments incorporating selective colonisation, the addition of metabolites, and the impacts of conventionalization are conceivable after germ-free subjects have been created. The optical transparency of larvae allows for *in-vivo* imaging of the zebrafish stomach during the early stages of development. This enables investigation of the localization and cross-species dynamics of fluorescently labelled bacterial species as well as GI function such as motility and barrier function. Additionally, the gut is simply cut open, making it possible to remove the microbiota for 16S sequencing [85, 86, 87]. Zebrafish have a considerable advantage over mammalian models when it comes to GI research because of their external fertilisation, rapid development also early transparency, which make investigating GI function in vivo much simpler. Before the larvae start feeding (with spontaneous motility appearing before 5 dpf), estimation of digestive transit, peristaltic rate along with general GI ontogeny can be conducted without the need for difficult or possibly variable-confounding surgical procedures [88]. Combining the two has drawn a lot of attention recently because of the growth in zebrafish-based GI research and the connection between ASD and GI disorders in non-zebrafish models.

It has been difficult to use mutant zebrafish to examine gene function, though. The fact that considerable amounts of mRNA generated by the targeted gene may still be present after genetic modifications intended to produce gene loss-of-function suggests that the goal may not have been fully attained [89]. In order to address this problem and facilitate ASD research utilizing zebrafish, "SFARI" (Simons Foundation Autism Research Initiative) is currently curating zebrafish lines with mutations in zebrafish paralogs of ASD risk genes. Gene loss-of-function is confirmed by monitoring mRNA or protein levels (where antibodies are available) rather than by evaluating phenotype. **Table 3** lists all the lines that SFARI has selected and is available at the "Zebrafish International Research Centre" (ZIRC).

TABLE 3: LIST OF GENES IN ZEBRA FISH ASD MODEL AND THEIR PHENOTYPIC FEATURES

Genes	Phenotypic Features	Reference
AT-rich interaction domain 1B (*arid1b*)	Body length reduction and altered expression of chondrogenic/osteogenic genes	91
Aristaless related homeobox (*arx*)	Neuronal changes and altered brain development	92
Activator of transcription and developmental regulator AUTS2 (*auts2*)	Reduced locomotor activity, undersized zebrafish bodies and heads, and microcephaly	93
Calcium voltage-gated channel subunit alpha1 C (*cacna1c*)	Cardiovascular changes	94
Centrosomal protein 41 (*cep41*)	Social behavior deficits and neuronal abnormalities	95
Chromodomain helicase DNA binding protein 2 (*chd2*)	Alterations in body structure, motor dysfunction and microcephaly	96
Chromodomain helicase DNA binding protein 8 (*chd8*)	reduced GI motility and macrocephaly	97,98
Contactin associated protein 2 (*cntnap2*)	Reduced forebrain GABAergic neurons at 4 dpf, microcephaly, and motor dysfunction	99
Catenin delta 2 (*ctnnd2*)	Body length reduction and different notochord changes	100
Dual specificity tyrosine phosphorylation regulated kinase 1A (*dyrk1a*)	Microcephaly, increased brain apoptosis, lower anxiety and freezing times, and deficiencies in social behaviors	101
Potassium inwardly rectifying channel subfamily J member 10 (*kcnj10*)	Developmental and motor changes	102
Lysine demethylase 6A (*kdm6a*)	Body length reduction and notochord changes	103,104
Methyl-CpG binding protein 2 (*mecp2*)	Immune response and neuronal changes	105,106
MET proto-oncogene, receptor tyrosine kinase	Death rates rising and neural impairments	107

(*met*)		
Myelin transcription factor 1 like (*myt1l*)	Reduction of oxytocin expression in preoptic neuroendocrine region	108
Neurobeachin (*nbea*)	Unusual reactivity to startle signals	109
Nuclear receptor subfamily 3 group C member 2 (*nr3c2*)	Changes in sleep and behavioral patterns	110
Oxytocin/neurophysin I prepropeptide (*oxt*)	Alterations in memory and oxytocin signaling	111
Reelin (*reln*)	Social behavior changes and disruption of the serotonin signaling pathway	112
Arginine-glutamic acid dipeptide repeats (*rere*)	defects in both vision and hearing, and an altered reaction to startling stimuli	113
SH3 and multiple ankyrin repeat domains 3 (*shank3*)	Impaired social affinity, hypoactivity, abnormal mid-hindbrain boundary, elevated CNS apoptosis, reduced GABAergic neurons, seizure-like behaviors	114,115
Synaptic Ras GTPase activating protein 1 (*syngap1*)	High death rate, developmental disorders, increased CNS apoptosis, motor impairment, and microcephaly	116

DISCUSSION & CONCLUSION: As behavioural assay development and data gathering progress, the value of zebrafish in behavioural neuroscience grows. The collecting of behavioural data is made more repeatable between studies and even labs by tools such as DanioVision, an observation box created exclusively for the monitoring of zebrafish larvae. Similar commercially accessible software programmes include Stytra [117], a free software programme made for tracking behavioural zebrafish investigations, and ZebraLab (Viewpoint), built for high-throughput assessment of embryos [118]. These and other technologies enable the highly measurable behavioural traits of zebrafish.

The effects of psychobiotics on autism spectrum disorder have been extensively studied in recent years. This biological therapy has potential, as has been demonstrated. More studies are needed to do that, and as is well known, it is not practical to conduct direct psychobioticexperiments on humans. Rodents were frequently employed in the past to study the effects of psychobiotics, but recent studies have shown that zebrafish models are considerably more suitable for use in clinical trials for human diseases like ASD. Despite the discovery of several ASD-related genes in the zebrafish genome, psychobiotic studies using zebra fish models have not yet shown any conclusive results. The effects of psychobiotics can be determined using a variety of behavioural assays. Rodent models are harder to find and far less accessible than zebrafish models. It is simple to maintain in laboratory settings. In order to have revolutionary effects in the field of neuroscience or in the treatment of mental health conditions like ASD, more and more study on the effects of psychobiotics in the zebrafish model is needed.

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