Genetic screening of autism, a neurodevelopmental disorder: A review

Ranjana Shaw¹, Tamalika Chakraborty*¹, Dipanjan Mandal²

¹Department of Biotechnology, Guru Nanak Institute of Pharmaceutical Science & Technology, Panihati, Kolkata-700114, West Bengal, India
²Department of Pharmacy, Guru Nanak Institute of Pharmaceutical Science & Technology, Panihati, Kolkata-700114, West Bengal, India

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ABSTRACT

Neurodevelopmental disorders are conditions that arise from aberrant encephalon magnification and development, are perennial, crippling illness. Autism spectrum disorder (autism) is a common neurodevelopmental disability characterized by motor, social, cognitive function, stereotypies. This study aimed to investigate the genetic constitution of autism, an animal model named zebrafish serves as an indispensable tool for this purpose. Zebrafish is a highly gregarious species that acts as a suitable animal model system that affects convivial functions such as autism. Since transparent embryos are developed externally in zebrafish, it enables to conduct pharmacological screens for recognition of minute molecules along with genetic manipulation, facilitated through the CRISPR/Cas9 gene-editing technologies, enabling the screening of the developing nervous system directly, large progenies, and substantial tractability. Mutational analysis of the genetic function has been used to suppress or express mutations in zebrafish homologs of human genes for the direct expression of human genes bearing mutations cognate to a neurodevelopmental disorder. Two areas of future research are addressed through the ease and relative speed of conducting experiments in zebrafish, which includes environmental factors contributing to disease onset, and screening for novel therapeutic compounds. This study found that zebrafish have become available for cell-based analysis and have been used for the prosperous modelling of autism. Continued innovations in zebrafish genetic implementations will continue to make it a captivating neurological disease model.

INTRODUCTION

Neurodevelopmental disorders have severe astringent effects on individuals, families, and society. Autism spectrum disorder (Autism) is a widespread neurodevelopmental disorder that involves problems with communication and behaviour. People with autism have trouble in communication, difficulties in expression, problems in learning, their skills might develop unevenly. Children show signs of autism usually from birth or before turning three, common symptoms range from lack of eye-to-eye contact, a limited range of interests in some subjects, continually speaking, such as repeating words, sensitive to some sounds, touches, smells, which seem
familiar to others, not listening to others, difficulty in understanding or using voices, facial expressions, verbalizing in a flat or robotic voice. The factors that fall within the spectrum of autism include:

Asperger’s syndrome
The child shows psychological issues and a restricted range of interests. But they do not have a language problem; in practice, in intelligence tests, they incline to score averagely.

Autistic disorder
People think of this disorder when they perceive the word “autism” relating to cognitive problems, social contact, and an autistic child often experience challenges in terms of communication.

Childhood disintegrative disorder
The infant grows typically for at least two years and unexpectedly loses some or most of its speech and social interaction abilities.

Chronic developmental disorder
These children exhibit autism traits, such as differences in social and communication skills.

It is unclear why autism happens, and it could be the difficulties in certain portions of the encephalon, perceiving sensory integration, and in interpreting language. Autism is more prevalent in males than in females. The investigation of the genetic substratum of autism is circumscribed by the size of the sample, scope, the efficiency of applied genomic technology. The data obtained from the single-nucleotide polymorphism microarray technique provides common variants under a genome-wide association study (GWAS) model along with the accession of large copy number variants (CNVs). Later, whole-exome sequencing (WES) is commonly used. The focus shifted to de novo and rare, inherited variants within the genome that participate in the protein-encoding functions (Turner and Eichler, 2019). Every breakthrough in technology offers incipient insights into the genetic screening of autism.

A necessary implement to study autism in animal models, the most commonly used are mice, zebrafish possessing several advantages, but are extravagant to maintain and are unapproachable for embryonic phase studies. Contrarily, zebrafish having minute body size, sizably voluminous brood size, optical transparency, which provides better constraints of animal models to conduct gene mapping and genome mutagenesis, exaggerated expression or suppression of protein, transgenesis, interpretation of embryo chimerically, cell transplantation, and screening, making this organism perfectly suitable for the molecular genetic screening of vertebrate neuropsychiatric disorders (Veldman and Lin, 2008). Visualization of the zebrafish encephalon neurotransmitter system when it exhibits a sophisticated repertoire of behaviours after five days of fertilization (Panula et al., 2006). Additionally, the nervous system in fish larvae allows circuit analysis (Burgess and Granato, 2007).

Comparing human and zebrafish encephalon’s and genetics
Zebrafish and humans’ nervous system is homogeneous, consisting of primary encephalon including diencephalon, telencephalon, cerebellum, the motor, and sensory components accompanying the peripheral nervous system. These enteric nervous systems function autonomously (Mueller and Wullimann, 2009). Many encephalon regions are relevant to 76-82% of conserved human disease genes present in zebrafish, and the remaining genes get duplicated averagely around 20-24% (Howe et al., 2013). Data obtained from human structural analysis correlate with the amygdala and habenula affective behaviour in zebrafish (Kaluff et al., 2014). Zebrafish possess all neuro mediators systems, including receptors, transporters, enzymes, alike in humans. A well-organized functional neuroendocrine system is shown in zebrafish that is homologous to humans. For instance, the cascade of hypothalamic-pituitary hormones in zebrafish shows stress responses that are mediated by cortisol via glucocorticoid receptors. In zebrafish, twenty-five pairs of chromosomes are present containing more than 26,000 protein-coding genes (Kaluff et al., 2014). Therefore, the genetic homology of zebrafish is relatively high, having 70 homologies with that of human genes (Kaluff et al., 2014). Random mutations are generated and to express encephalon behaviours and functions in zebrafish various novel genes were discovered. Various genetic implements also exist for this purpose. Clustered Regularly Interspaced Short Palindromic Repeats’ (CRISPR) system functionally characterize a target zebrafish gene of interest through over expression or selective target/suppression (Heintze et al., 2013; Hruscha et al., 2013). Being advantageous, zebrafish models possess some challenges in modelling disease. Various human encephalon studies determine certain encephalon regions having a similar function in respect to humans still need to be done. Zebrafish have retained gene duplicates (Glusauer and Neuhauss, 2014) allows both sub-functionalization of pleiotropic phenotypes (advantages) and genetic redundancy (disadvantages) for the successful creation of disease models (Kozol et al., 2016).
Utilization of Zebrafish to study Autism

The behavioural spectrum of zebrafish is limited and cannot recapitulate all facets of human behaviour that are affected by autism but are useful for genetic screening. However, zebrafish models provide new insights into the in vivo function of the autism-implied genes (Tropepe and Sive, 2003). This is so because it can externally produce larvae to a greater extent, which can be used for genetic and molecular screens (Mathur and Guo, 2010). Single-cell analysis of transparent larvae enables observations of developed encephalon inside living embryos. Social interaction in zebrafish is accessed by a gene homologous of zebrafish involved in autism, as shown in Figure 1 (Vernier et al., 2012).

One study focusses that one percent of autism occurs due to a single area of the 16th chromosome gets deleted, which includes almost 30 genes (Eichler and Zimmerman, 2008) of which 25 genes have direct counterparts of zebrafish (Barbazuk, 2000), to recognize the normal encephalon structural and functional genes, morpholino antisense oligonucleotides (Nasevicius and Ekker, 2000) are used accompanying via ways of means of interactions between genes. The physiological functions of Sushi domain-containing protein 4 (SUSD4) provide the first perception that genes get deleted in autism patients, accessed through the zebrafish morpholino knockdown experiments. The central nervous system (CNS) of humans, mice, zebrafish shows a high expression of SUSD4 (Vernier et al., 2012). Autism is associated with the changes in the structure of the cerebellum, the disrupted cerebellar gene expression (Winter et al., 2008). The developmental basis of autism is understood through the function of the Met (proto-oncogene associated with metastatic cellular cancer)/HGF (hepatocyte growth factor) shows signals utilized in the development of the cerebellum (Eisen et al., 2009) as a result revealing that for cerebellar morphogenesis, consisting of the average growth and specification of the type of cell, Met signalling is crucial which performs a critical role in hind brain cell migration.

Humans’ Neuroligin 3 and Neuroligin 4 genes possess mutations that show connectivity for autism and mental retardation (Rissone et al., 2010). In synaptic function and maturation, neureligins are involved along with neurexins (Hirata et al., 2011). Zebrafish offers an extremely good possibility to examine the function of expressed neureligins throughout the nervous system of zebrafish, enlightening the developmental basis of autism. Studies in zebrafish have revealed that human homologs are very similar to the seven genes in the zebrafish neuroligin family (Teles et al., 2016). It suggests that vigorous evolutionary pressure is subjected to preserve the functions of these genes.

One study focusses on the gastrointestinal distress, frequent comorbidity experienced by individuals with autism (Liu et al., 2018). For this purpose, a zebrafish shank3 mutant model was created to analyze a causal relation among shank3 deprivation mutations and digestive tract (DT) impairment. SHANK3 is present in the central nervous system where it acts as a scaffolding protein, regulating synaptic development, actin polymerization, dendritic spine formation. It also plays a role in gastrointestinal host/symbiont interactions, zinc metabolism, intestinal barrier function. This model shows results in genome duplication, yielding shank3a, shank3b paralogues (Kozol et al., 2015). In the C terminal, zebrafish and shank3a prolinerich domains produce frameshift mutations (18th chromosome), shank3b (4th chromosome) through CRISPR/Cas9 technologies to model shank3 mutations connected to autism. The online software CHOPCHOP plays a vital role in the generation of site-specific CRISPR-Cas9 single-stranded guide RNAs (sgRNAs). In vitro transcription of sgRNA and Cas9 proteins occurred followed by some incubation steps, the genomic DNA was then screened for mutations. For the screening purpose, polymerase chain reaction (PCR) is utilized. Frameshift mutations in shank3a and shank3b have been done through the CRISPR-Cas9/sgRNA injections. In shank3, the largest exon is targeted through both by encoding a sizeable C-terminal region, having numerous protein interaction domains. These mutations are associated with autism and can be screened through the utility of CRISPR-CAS9 gene-editing technologies.

The success of zebrafish models in autism studies

The repertoire of zebrafish behaviours, social nature, and its dependency on vision is strong since it is diurnal species, makes it an excellent model system for the encephalon function assessment and dysfunction (Shams et al., 2018). Zebrafish’s reliance is characterized as an essential sensory modality. Zebrafish is an excellent organism for high-throughput screens. Optogenetic models characterize zebrafish models, and transgenic zebrafish fluorescence-based screens permit biological processes data visualization of encephalon disorders and cellular mechanisms (Shimada et al., 2012). Also the cost of in vivo screening of a drug in zebrafish is very cheap in comparison to other animal models, thus providing an affordable assessment of genetic or pharmacological modifier
Various internal and external signals can evoke behavioral responses that get altered by an impaired neurological condition in autism

libraries to a greater extent (Lieschke and Currie, 2007). Zebrafish models also enable the rapid identification of candidate genes or active compounds for efficient screening. Zebrafish mutants also enable risk gene function throughout for the improvement from embryonic stages via adulthood. It is essential to autism, where risk genes within the human encephalon are highly expressed during embryonic and fetal stages (Sakai et al., 2018).

The limitations of zebrafish models in autism studies
Besides possessing numerous advantages, the zebrafish model possesses some limitations. The paucity of well-characterized inbred strains is one of the recognized limitations in zebrafish genetics (Sison et al., 2006). Besides that, there are many strains outbred of them, having unclear breeding history, showing only partial inbreeding. The number of available strains for screening purposes is limited; this situation is not favourable as it is limiting the main resource. Despite being a highly social animal, there is no clarity between behaviours of the same and complex human disease autism. For instance, individuals with autism face a concentration deficit which conducts problems in reading face countenance (Webb et al., 2012; Hosozawa et al., 2012). It is yet not disclosed whether the fish requires similar attention processing or not (Riby et al., 2012). Increased gene duplication in zebrafish has increased the challenge of investigating their function (Kassahn et al., 2009). The investigation of the environmental modulation of autism-like behaviour in zebrafish has not yet been done.

Future Directions
Zebrafish models elicit a successful attempt in the modelling of many human encephalon disorders. The zebrafish model advances towards an excellent line of neuroscience research in the future, offering more advantages in the fields of targeted expression, multigenic analysis, chemical genetics. Future studies of zebrafish models need to answer many of the issues, one of them is despite being a highly social species, it remains unclear if a deficiency in zebrafish shoaling behavioural repertoire may be compared to a complex human neurodevelopmental disorder such as autism. Another deficit associated with autism is a shift in stimulus-driven attention that can conduct problems in decoding face countenance. Further research is needed to address whether fish shoaling involves the processing of similar attention or not. The translational significance of the phenotypes linked to concen-
tration and self-control in zebrafish needs to be addressed in-depth (Parker et al., 2013; Echevarria et al., 2011). Furthermore, zebrafish studies should be continued to offer new perceptions into fundamental, progressively preserved neuropathology of stress-associated neurodevelopmental disorder.

CONCLUSIONS

Behavioural neuroscience deals with many experiments with different model organisms, in certain particular species, similar to humans, having sufficient physiological complexity, similarity, such as zebrafish are urgently needed to model neurodevelopmental disorders. Similar to humans, zebrafish acquires environmental information employing specialized sensory organs like the eye, ear, olfactory system. Nervous system process this information to generate a repertoire of behaviours. Therefore, zebrafish become amenable for screening and analyzing at the cellular level, were used for the successful modelling of neurodevelopmental disorder. The continuing advancement of zebrafish genetic techniques will continue to make it an appealing model for neurological disease. Furthermore, standard assays are combined for comparison of models with newer methods, which would require an understanding of cellular, molecular, neurological conditions. Thus, zebrafish serves as a unique genetically tractable model system, where molecular, cellular, development mechanisms underlying neuropsychiatric disorders can be easily understood and will lead to consortia of study groups using several animal models for circuit analysis implicit neurodevelopmental disorder.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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