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Zebrafish: A Versatile Animal Model in Biomedical Research

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Abstract:
Zebrafish (Danio rerio) has emerged as a powerful animal model in biomedical research, offering a unique combination of genetic tractability, rapid development, and conserved gene function with humans. This review highlights the significance of zebrafish in understanding various human diseases, including neurodegenerative disorders, metabolic disorders, cancer, and more. The zebrafish genome, with over 70% similarity to the human genome, allows for the modelling of human diseases with high fidelity. The ease of genetic manipulation, high fecundity, and low maintenance costs make zebrafish an attractive model for large-scale genetic screens and drug discovery. Zebrafish models of neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, and Huntington's disease, have been developed, providing insights into disease mechanisms and potential therapeutic targets. Similarly, zebrafish models of metabolic disorders, including diabetes, dyslipidaemia, and atherosclerosis, have been established, enabling the study of disease pathogenesis and prevention strategies.The zebrafish has also been used to model various types of cancer, including leukaemia, melanoma, and pancreatic cancer, facilitating the discovery of novel oncogenes and tumor suppressors. The transparent nature of zebrafish embryos and larvae allows for in vivo imaging of cancer cells, enabling the study of cancer cell behavior and response to therapy.
Furthermore, zebrafish has been used to investigate brain-to-organ communication, cell transplantation, and cell-cell interactions, providing insights into the complex interactions between different tissues and organs. The development of novel tools, such as CRISPR/Cas9 genome editing and single-cell RNA sequencing, has further expanded the capabilities of zebrafish research.

Keywords: Zebrafish, applications, animal model, biology,

1. INTRODUCTION:

The zebrafish, or Danio rerio, is an incredibly useful, quick, and efficient model system for researching the developmental biology of vertebrates. When zebrafish were first employed as a contemporary model organism in the early 1980s, numerous new methods for observing and altering these early embryonic processes were developed. Because zebrafish and other vertebrates including humans have a high degree of genetic and molecular similarities, many important discoveries made in zebrafish development have applications to humans [1]. This review summaries the applications of the zebrafish in various models.
Many animal species are useful as experimental models in the advancement of biomedical science. The

validity and consistency of study findings from in-vitro or rodent investigations are provided by animal models.

An increasingly common animal model used in biomedical research is the zebrafish. Characteristics of Zebrafish, formerly known as Brachydanio rerio, zebrafish, or Danio rerio in Latin, are small tropical freshwater fish that are native to the Ganges River and its tributaries in northern India [2]. Compared to model organisms of mammalian vertebrates like mice and rats, zebrafish provide some noteworthy advantages.

Zebrafish embryos rapidly develop externally, and this process is visible to the naked eye [3]. Streisinger and associates first created zebrafish in the early 1980s as an animal model for genetic research [1]. Additionally, Humans and zebrafish have a high degree of genomic structure (~70%), with each human gene having at least one clear zebrafish orthologous, in contrast to 80% for mouse orthodox. This has simplified the process of studying human genetic disorders using zebrafish. Thanks to recent advancements in next-generation sequencing (NGS) and the need for tailored therapy, zebrafish are increasingly being utilized to discover the causal relationships between the genotype and phenotype of numerous human diseases [4, 5].

Fig. 1 Photos of Zebrafish (Danio rerio)

2. ZEBRAFISH BACKGROUND:

Zebrafish have been a popular pet for a long time. Its susceptibility to large-scale forward genetic screening led to a major spike in research about eight years ago. Large-scale genetic screenings like this have previously only been done on invertebrates like yeast, worms, and flies. The ability to use a forward genetic approach to comprehend mechanisms unique to vertebrates that impact development and illness has been made possible by zebrafish. Thousands of mutations affecting organogenesis, physiology, and behaviour have been produced in the last 10 years. These mutations have proven to be a rich source of information about the relationships between genes and functions. Since several new approaches have been developed in recent years, zebrafish have become a much more useful creature for experiments. The trans-National Institutes of Health Zebra fish Genome Initiative has thoroughly annotated the zebrafish genome, which has now been entirely sequenced by the Sanger Center. There are several DNA microarrays available for expression profiling tests as well as whole zebra fish cDNA sets. Another quick method for analysing gene expression is whole-embryo in situ hybridization. Using antisense morpholino

oligonucleotides, gene function in zebrafish can be quickly and reliably investigated. In addition, techniques for transgenic line generation, targeted mutations (reverse genetics), and nuclear transfer cloning have been developed. Thanks to these technologies, zebrafish have become the preferred subject of study for many academics, as seen by the substantial increase in zebrafish publications in recent years. The quantity of PubMed references pertaining to zebrafish has increased over the past 10 years by over ten times, and in the previous five years, it has increased by almost three times [6].

ZEBRA FISH'S IMPORTANCE AS AN ANIMAL MODEL

Contribute to the current zebrafish era in biomedical research by using zebrafish as a biomedical model [7]. A fully sequenced genome, simplicity of genome manipulation, high fecundity, a short generation time (about three months), a quick 24-hour embryonic development period, and external fertilization are only a few of the advantageous characteristics of zebrafish. More than 10,000 protein-coding gene mutants have been made, and numerous transgenic lines of zebrafish have been developed for the purpose of studying human diseases [8].

For genetic mapping investigations and trials requiring high sample numbers, the availability of children is very beneficial. Compared to the animal facilities needed for mammals, zebrafish may be produced and kept in high-density tank systems with far less area and expense. Zebrafish were first used as an experimental model by researchers because of their characteristics.

Table No 1: Different applications of Zebrafish

Sl.No	Types of fish	Model	Mechanism of action	Reference
1	Zebra Fish (Danio rerio)	Neurodegenerative disease (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine MPTP) Parkinson's Disease)	Neuroprotection • α-syncline degradation. • A rise in the genes associated with antioxidants (sod1, gss, gpx4a, gclm, and cat). • Due to the antioxidation mechanism, demonstrated anti-PD action.	[9]
2	Zebrafish (Danio rerio)	Neurodegenerative disease (astaxanthin in zebrafish with AD related with CVD)	Neuroprotection • Decreases in MMP-13 activity, acetyl cholinesterase activity, and amyloid beta-peptide aggregation.	[10]
3	Zebrafish (Danio rerio)	Neurodegenerative illness (3-HD in adult zebrafish induced by NP)	• Reduced reactive astrocytosis, NMDA Antagonist • Enhanced expression of the BDNF/tropomyosin-related kinase-B receptor and enhanced vascular density. • Found change in body weight and behaviours,	[11]

			• Reduction of neuro inflammation through reduction of IL-1β and TNF-α levels. less injury to neurons.	
4	Zebrafish (Danio rerio)	Metabolic disorder (fructose-mediated glycation with low-density lipoprotein (LDL))	• Controlled metabolites connected to the pathways for lipid metabolism, amino acid metabolism, and glycolysis. transcription of a few genes involved in fat and glycolysis metabolism	[12]
5	Zebrafish (Danio rerio)	Metabolic disorder (inflammation through lipopolysaccharide (LPS) injection.)	• A decrease in the expression of pro-inflammatory cytokines in LPS-stimulated zebrafish, such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and interleukin-1 beta (IL-1β). Inflamed zebrafish treated with PSCP prophylactically experienced reduced skin hemorrhage, normalized breathing, and avoided caudal fin loss.	[13]
6	Zebrafish (Danio rerio)	Endocrine system	• The blood levels of glucose, AST, ALT, and ALP were lowered by metformin and silymarin. The fish body needs to raise the absorption level by increasing the amount of acidic goblet cells, which acidifies the environment in the stomach tracts, because a diabetic's weakly absorbs nutrients.	[14]
7	Zebrafish (Danio rerio)	Cancer (xenotransplantation of MCF-7 breast cancer cells and human JF 305 pancreatic cancer cells into zebrafish)	Anti-cancer role • Through apoptosis, DNA strand breaks, anti-angiogenesis, and the induction of ROS generation. It has been shown that increased ROS production damages major biological molecules, such as DNA, resulting in apoptosis and DNA strand breaks.	[15]

			• Caspases 3/7 interacts with caspase 8 and 9 and is in charge of the proteolytic cleavage of several proteins during apoptosis. The cancer cell xenotransplanted zebrafish treated with furanodiene showed a significant rise in caspases 8, 9, and 3/7, indicating that furanodiene-induced zebrafish apoptosis is dependent on both caspase 8 and caspase 9, which results in cancer cell death.	
8	Zebrafish (Danio rerio)	Hepatoprotective (Acetaminophen PAP-induced liver injury in zebrafish)	Hepatoprotective Effect • By controlling targets such as phosphatidylinositol 3-kinase (PI3K), matrix metallopeptidase 9 (MMP9), matrix metallopeptidase 2 (MMP2), and tumor necrosis factor (TNF). The apoptotic signalling pathway mediated by PI3K/AKT and extracellular matrix remolding genes might be reversed by FA, according to PCR data.	[16]

Metabolic disorders:

Zebrafish are commonly used as animal models in metabolism research. A person who consumes large amounts of calories, leads a sedentary lifestyle, and has a family history of metabolic diseases is more likely to have risk factors such as low HDL, high triglycerides, high blood glucose, high blood pressure, and abdominal obesity [17]. Metabolic disorders such as fatty liver disease, diabetes, and stroke might arise as a consequence of these issues. An imbalance between energy expenditure and dietary intake may potentially be the cause of them [18]. Apart from general similarities, zebrafish metabolism has unique characteristics. Zebrafish embryos consume yolk throughout the first five days of their existence in order to sustain growth and avert famine. Feeding-to-fasting transition, which takes place 5-7 days after conception, has been used to acquire mechanistic insights into metabolic homeostasis during calorie deprivation [19, 20]. Other distinguishing features of zebrafish include the composition and development of their adipose tissue. Unlike mammals, zebrafish are poikilothermic, which means they don't seem to require brown adipose tissue. Eight days after birth is when the first adipocyte is discovered, suggesting that adipose development occurs later in life [21]. Interestingly, research on the role of adipose tissue in the development of metabolic illnesses may also be possible in late adipogenesisIt is possible to adequately build modelling metabolism to simulate human illnesses during the larval phase. Similarly, [22] Metabolic illnesses in adults can be simulated to examine phenotypic references in the presence of the major

metabolic organs. There have previously been discussions on the modelling of many metabolic diseases as well as the numerous metabolic similarities and variances between humans and zebrafish [23, 24].

Zebrafish models of Parkinson's disease:

In the zebrafish model, Parkinson's disease (PD) is by far the most well-established neurodegenerative sickness and movement disorder. A recent study highlighted the PD model's promise as an animal model to help discover treatments [25]. Zebra fish have a high degree of gene conservation related to Parkinson's disease (PD) and are sensitive to drugs linked to PD risk, which has led to the creation of numerous genetic, transgenic, and chemically induced models of the disease. The diencephalic dopaminergic cluster in the posterior tuberculin of zebrafish's acts similarly to the mammalian SNpc, despite the absence of dopaminergic neurons in the medial brain. Furthermore, there is a considerable similarity between the serotonergic and histaminergic systems of zebrafish and mammals [26]. The zebrafish model, while not a perfect replica of the disease, can be a valuable tool for researching hypokinetic disorders associated with Parkinson's disease. Parkinson's disease (PD) patients may have clinical features similar to bradykinesia due to dopaminergic cell abnormalities in zebrafish, as will be demonstrated below [27].

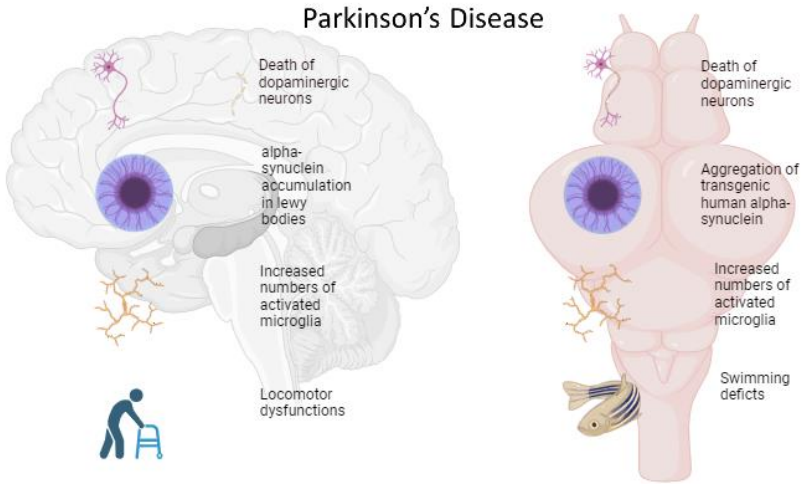

Figure 2. Mechanism of PD in human and zebrafish

Table No 2: Genetic Zebrafish models of PD

Methods	DA neuron loss	Other pathologies	Motor deficits	Other phenotypes
PINK1 MO knockdown	Yes	ROS accumulation	Yes–Impaired TEER	Morphological Deformities. Increased mortality.
Parkin MO knockdown	No	Increased susceptibility To proteotoxic stress	Not reported	-
LRRK2 MO knockdown	Yes	Synuclein aggregation	Not reported	Morphological Deformities.

PINK1 MO knockdown, Micro array analysis	Not reported	Significant alteration of 177genes. Increased ROS levels	Not reported	Reduced heart rate. Increased Erythropoiesis.
Parkin Mok nockdown	Yes	Reduced mitochondrial Activity.	No	-
FBXO7 MO knockdown	Yes	-	Yes–Reduced swim velocity	Morphological Deformities. Increased mortality.

Table No 3: Chemical Zebrafish models of PD.

Methods	DA neuron loss	Other pathologies	Motor deficits	Other phenotypes
MPTP	Not reported	-	Yes, there was a decrease in swim movement and distance, a decrease in crossings, and an increase in the number and length of freezing bouts.	-
6-OHDA	Not reported	-	Yes. There was a decrease in swim distance, speed, and maximum acceleration; there was also an increase in absolute turn angle and immobility time.	Reduced head and total Length.
Paraquat	Not reported	-	Yes—decreased line crossings, decreased swimming distance and speed, and impaired motor coordination	-
MPTP	Yes	-	Definitely—less swimming	Adults: darker pigmentation and respiratory dysfunction
Rotenone	Not documented, but caused a phenotype of brain death	decreased mitochondrial membrane potential in skeletal muscle	Yes–Reduced responsiveness	Developmental deformities

Zebrafish as a model for Dyslipidaemia and Atherosclerosis:

Elevations in triglycerides, cholesterol, or high-density lipoprotein cholesterol caused dyslipidaemia, which paved the way for the onset of atherosclerosis. Given that the nutritional needs of zebrafish are well understood, a number of researchers have created several models by altering the typical diet for example, by giving zebrafish a high-fat diet that causes obesity, hyperglycaemia, and dyslipidaemia in order to put the fish through metabolic stress. The symptoms of atherosclerosis in humans are strikingly comparable to the histological alterations seen in zebrafish on high cholesterol diets. The creation of a diet high in cholesterol is crucial for the investigation of dyslipidaemia [28, 29]. Outlined the steps of lipid and lipoprotein metabolism using the metabolism of the embryonic zebrafish yolk and concluded that the system's ability to produce lipoproteins was a prerequisite for the circulatory system's ability to absorb exogenous fatty acids [30].

Zebrafish as a Type 2 Diabetes Mellitus and Glucose Metabolism Model:

The main reason of the development of diabetes mellitus is a shortage of insulin, which is caused by the pancreatic β-cells' inability to make enough insulin. Zebrafish and humans both make use of these identical systems and capabilities. Exposure of zebrafish to diets high in fat and calories soon results in obesity and obesity-related diseases, as well as activating metabolic pathways that are comparable to those found in humans. The pancreas produces insulin in response to glucose availability in the diet, and gluconeogenesis is prevented by down regulating genes involved in the mechanism. Glucagon triggers the process of gluconeogenesis when there is no glucose present in the circulation [31]. Showed that when zebrafish are immersed in a high-glucose solution (111 mM) for 14 days, they may experience hyperglycaemia, decrease in mRNA for insulin receptors in the muscle, and increase in froctosamine (glycated protein) from the eyes [32]. We developed a zebrafish model of type 2 diabetes mellitus by providing them with an excessive amount of food, 408 calories per fish per day. Gene expression profiling in the liver and pancreas revealed a similar mechanism for the establishment of type 2 diabetes mellitus in humans and zebrafish. Research on the relationship between age and type 2 diabetes mellitus revealed that young zebrafish (4 to 11 months old) acquired hyperglycaemia more slowly than older zebrafish with increasing glucose concentrations [33]. By submerging zebrafish embryos in a glucose solution, the amount of glucose in the organs responsible for maintaining homeostasis can be raised. Shown that giving adult zebrafish 1% glucose for a full day might increase their blood glucose levels to 400 mg/dL. In the two transgenic models of insulin resistance that were created, transgenic expression of a dominant-negative IGF-I receptor in skeletal muscle resulted in skeletal muscle insulin resistance [34]. In the second model, the insulin receptor gene in the liver was precisely knocked down using CRISPR/Cas9, resulting in insulin resistance [35]. These results showed that zebrafish are a useful model to employ when studying human disorders caused by glucose [36]. In addition, a model for hyperinsulemia was produced by infusing human recombinant insulin into zebrafish larvae. These studies showed that tyrosine phosphatase non-receptor type 6 is a negative immunological modulator protein that is more prevalent in insulin-resistant larvae. According to recent studies, mutant zebrafish with a knockout in the genes for insulin receptors a and b showed symptoms, such as hyperglycaemia, decreased growth hormone signalling, increased visceral adiposity, and the development of fatty liver, when fed a high-carbohydrate (41%) diet. These symptoms are similar to those of human lipodystrophy disease. Zebrafish's glucose content can be measured using two portable glucose meters designed for diabetics [37, 38]. Moreover, intraperitoneal and postprandial glucose tolerance testing can be carried out during fasting. Numerous methods, including

semi-quantitative dot blotting, insulin antibody immunostaining, and qPCR determination of the insulin mRNA expression level, can be used to measure the in Insulin sensitivity in hyperglycaemic zebrafish can also be assessed by intraperitoneal administration. Insulin levels in zebrafish [39, 40]. In hyperglycaemic zebrafish, intraperitoneal injection of insulin can also be used to measure insulin sensitivity [41].

Zebrafish models of Parkinson's disease:

The most common cause of dementia is a persistent neurological disease. The two main characteristics of AD are extracellular amyloid (A) deposits, which are made from cleaved amyloid precursor protein (APP), and internal neurofibrillary tangles (NFTs), which are made of aggregated hyper phosphorylated tau proteins. The disease causes progressive atrophy of the parietal and hippocampal brain regions [42]. GWAS (genome wide association studies) has identified numerous high-risk loci genes associated in the control of immunological responses, raising the possibility that microglia play a role in the etiology of AD [43, 44, 45]. It's interesting to remember that A plaques exist in the brain even before cognitive decline. Nonetheless, NFTs have been connected to cell death, neurodegeneration, and cognitive decline [46, 47, 48]. Recent PET imaging investigations and meta-analyses of published biomarker data demonstrate a strong association between total tau levels in blood and cerebrospinal fluid and cognitive impairment in AD patients [49, 50]

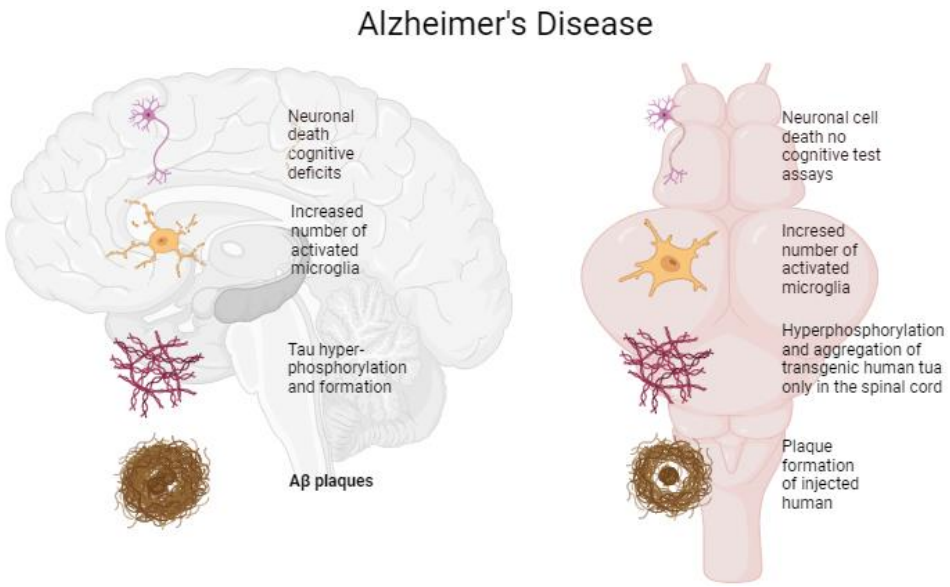

Figure 3. Mechanism of AD in human and Zebrafish

Table no 4: Zebrafish models of tauopathy.

Method(s)	Target of tau phosphorylation	NFT formation	Other phenotype
MAP-Tau4R mutation	Enolase-2promoter–Neurons	Yes	-
Tau P301L mutation	her4.1promoter–NPCs(with radial glial identity)and neurons	No–Investigated in adult zebrafish	-

TauP301Lmutation	PanN:Gal4VP15driver Pan-neuronal	Yes	Increased loss of neural cells. Compromised activity of proteasomes.
TauP301Lmutation	HuC promoter–Neurons	Yes	Increased neuronal cell death.
FTDP-17mutation	GATA-2promoter–Neurons	Yes	Cytoskeletal filament disruption in the cell axon

Table No 5: Zebrafish models of Aβ toxicity.

Method(s)	Aβ aggregation	Normal cell death	NSPC Proliferation and neurogenesis	Other phenotype
Human Aβ42 (ventricular injections)	Yes	Yes	Yes	Creation of Aβ sheets inside cells. Reduced learnt behaviour and impaired conditioning
Human TR- Aβ 42 (ventricular injections)	Yes	Yes	Yes	Higher level of microglia activation. Higher levels of synaptic degeneration.
Aβ 1–42(ventricular injections)	Not reported	Yes	Not reported	Elevated phosphorylation of tau. impeded evasion of Unpleasant stimuli.
Human A 42 (Expression in Melanophores under Mitfa promoter)	Not reported	Not reported	Not reported	Unusual pattern and skin pigmentation loss

Zebrafish models of Huntington's disease:

Numerous groups have investigated the effects of HTT depletion on zebrafish early development in an effort to identify the physiological role of HTT. Zebrafish contains a homolog of human HTT that is 3,121 amino acids and 70% comparable to mammalian HTT; however, it only contains 4 glutamines, compared to up to 35 in human HTT and up to 7 in mice [51]. Zebrafish brains, like human brains, show extensive expression of HTT, which is necessary for the preplacadel and telencephalic progenitor cell formation [52, 53]. The telencephalon of zebrafish may be the anatomical counterpart of the striatum in mammals, according to certain theories [54]. Furthermore, the loss of zebrafish tissue produced from placodes, including lateral line sensory neurons and olfactory neurons, is identical to the clinical observations of increasing olfactory impairments in HD patients [55].

We investigated the effects of HTT deletion on several brain regions in zebrafish, concentrating on CNS regions that express HTT [56]. Reduced expression of the genes (six1, dlx3b, and emx3) that are normally expressed in the anterior most part of the neural plate suggests that the development of that region was

hampered by the inhibition of HTT mRNA translation. The anterior neural plate induces a variety of forebrain structures, including telencephalic precursors and preplacadel cells [57, 58, 59], during a different re-examination. Investigated HTT's function in the neural tube's development. HTT is required for homotypic connections between neuroepithelial cells, according to concurrent research utilizing HTT-depleted zebrafish embryos and HTT-null mouse embryonic stem cells [60]. Inhibition of HTT translation hinders rosette formation and neurulation, just like N-Cadherin ablation does [61]. Furthermore, it was shown that 24 hours after fertilization, the disruption of the apical marker ZO-1, which is required for proper synthesis and distribution, resulted in misplaced cells in the diencephalic neural tube and cellular aggregates in the brain ventricles [60]. Furthermore, compared to controls, HTT morphants displayed altered ventricular space and reduced cephalic regions. Remarkably, the effects of HTT depletion were restricted to alar regions of the forebrain, with cells remaining organized in more basal places; this is comparable to N-Cadherin mutants. HTT knockdown also caused elytroid and ubiquitous transferrin receptor transcript levels to increase, maternal iron reserves in the yolk to be depleted, and blood haemoglobin levels to decrease [61]. The findings suggested that HTT is involved in the release of iron from endocytic compartments into the cytosol because it works downstream of transferrin receptor-mediated iron endocytosis. This is in line with reports of iron insufficiency and iron metabolism Dysregulation in HD patients [62].

Table no 6: Zebrafish models of Huntington's pathology.

Method	Neuronal loss	Impaired metabolism	Motor deficits	Other phenotype
AMO knockdown	Yes	Reduced BDNF levels.	Not reported	Morphological Deformities. Increased mortality.
AMO knockdown	Too early	Not reported	Not reported	Impaired brain Development. Morphological Deformities.
AMO knockdown	Not reported	IncreasedADAM10 Activity. Increased Cadherin Cleavage.	Not reported	Impaired brain Development.
AMO knockdown	Not reported	Impaired iron metabolism. Reduced haemoglobin production.	Not reported	Developmental Retardation and morphological Deformities.
4Q,25Q,and102Q polyQexpansion	Yes–Only in 102Q	Not reported	Not reported	Morphological Deformities and Increased mortality (102Q).

CRISPR/Cas9deletion	No	No	Not reported	Reduced fitness and Survival in adulthood.

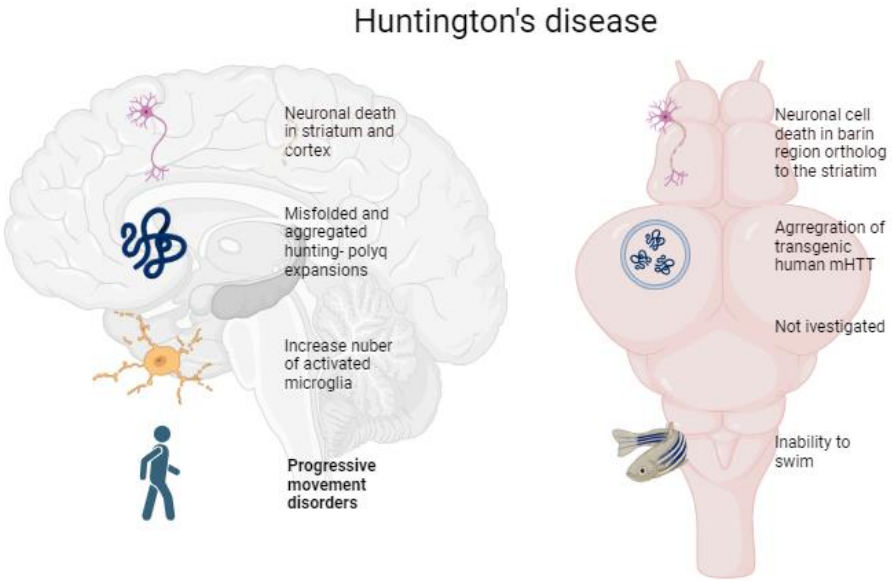

Figure 4. Mechanism of HTT in human and zebrafish

Microarray Tools and the Zebrafish Genome Project:

Together with the zebrafish research community, the Sanger Centre is sequencing the zebrafish genome. With five and a half times the coverage, the most recent version, April 2007 Zv7, has about 45,000 predicted genes. This invaluable resource provides DNA sequence information for genetic mapping studies along with data mining tools for the identification of novel and conserved genes. Many gene microarrays have also been developed in parallel to investigate the expression of hundreds of genes in both experimental and natural environments. We have previously used these tools, along with a few others, to study changes in gene expression in cloche mutant embryos lacking blood cell lineages and endothelium [63, 64, 65]. These studies supported the significance of genes that were previously known to exist and also identified a large number of additional genes linked in the development of these tissues. It was found that zebrafish myeloid and endothelial cell development depends on a particular gene, an antes family transcription factor [66, 67]. A new study demonstrating the significance of genomic data, microarray analysis, and gene function conservation between fish and mammals shows that the mammalian homolog, ER71, is a crucial facilitator of these same processes in mice [68].

In-situ hybridization analysis of gene expression:

Zebrafish embryos can be utilized for RNA in situ hybridization (ISH) to study endogenous gene expression across the entire embryo because of their developmental transparency There are several techniques for staining embryos by in situ hybridization (ISH); the most widely used method entails hybridizing the embryo with digoxigenin-labeled antisense RNA probes, staining the embryo with an anti-digoxigenin antibody conjugated to alkaline phosphatase, and then catalysing an enzymatic reaction that yields a fluorescent product or collared precipitate [69]. This technique provides spatiotemporal

information about the expression of the gene(s) of interest and can be used to identify interactions between different genes when combined with whole-mount immunostaining and double ISH or ISH. It is possible to use this method as an effective forward genetic screener [70].

Zebrafish as a model for biomedical research: Brain-to-organ communication:

The hypothalamic-pituitary-gonadal (HPG) axis, which recognizes various endocrine glands as separate entities, is activated by the complex interactions that occur during the dynamic process of human puberty. Growth and regulation of many body functions, including reproduction, depend on the HPG axis [71]. The brain's hypothalamus releases gonadotropin-releasing hormone (GnRH), which circulates and binds to receptors on the secretory cells of the adenohypophysis through the anterior pituitary hypophyseal portal system [72]. In response to GnRH activation, these cells produce luteinizing hormone and follicle-stimulating hormone into the bloodstream [73]. As a result, an adolescent develops into a fully developed adult with a sexually reproducing body [74]. A genetic disorder called Kallmann syndrome (KS) prevents a person from reaching puberty. In a study showing that the WDR11 gene mutation is involved in KS pathogenicity, the zebrafish WDR11 gene was shown to be expressed in the brain region, suggesting a potential involvement for WDR11 EMX1 protein interaction [75]. The adult zebrafish brain's addition and acute inflammation following trauma cause a restorative response. The leukotriene C4 (LTC4)–cysteine leukotriene receptor 1 (cysltr1) pathway is both required and sufficient for enhanced proliferation and neurogenesis. A ligand of CysLT1, LTC4, interacts with a receptor on radial glial cells in the zebrafish brain called Cysltr1 [76]. Cysltr1 was shown to be expressed greater on radial glial cells after traumatic brain injury, suggesting potential contact between components of the inflammatory response and the central nervous system [77]. The family of enzymes known as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) produces reactive oxygen species in response to various extracellular cues. It was found that the NOX family member dual oxidase (DUOX) is a thyroid NADPH oxidase. DUOX2 mutations have been identified in human children with congenital hypothyroidism. Recently, it was demonstrated that in addition to growth retardation and goitres in the thyroid glands, duo knockout zebrafish also show abnormalities in their anxiety response and social interaction [78]. These results suggest that dual knockout zebrafish can be a valuable animal model for studying thyroid development and related neurological disorders like intellectual impairment and autism. Gastrointestinal problems include diarrhoea, constipation, and abdominal pain, and a considerable percentage of children diagnosed with ASD are known to experience them. Recent studies on the brain-gut axis have also shown that environmental cues can be derived from interactions with host-associated microbial populations. These interactions might happen indirectly through the immune, metabolic, or endocrine systems, or directly through microbial metabolites. The gut emits chemical signals to help the brain and gut communicate during moments of anxiety, depression, cognitive dysfunction, or autism spectrum disease (ASD) [79]. Furthermore, by manipulating external stimuli and intrinsic signalling pathways in resident gut microbes, β-catenin is stabilized, promoting cell proliferation in intestinal epithelial cells [80].

Cell Transplantation and Cell-Cell Interactions:

More than ten years ago, a method for using cell transplantation to create chimeric zebrafish embryos was discovered [81]. Cell-autonomous and non-autonomous genes, cell fate throughout development, the functional properties of signalling molecules, cell behavior analysis, and other topics have all been explored using this technique. An experimentally modified cell or cells are grafted into wild-type embryos

at the late blastula stage, and the development of the resulting chimera fish is monitored. Using fluorescent dextran conjugates to designate transplanted cells is the most common technique. To determine the fate of the cells, immunohistochemical staining (ISH), anatomical location, or both are utilized. Using wild-type cells in an embryo that has undergone experimental modification, the opposite experiment can likewise be carried out. Furthermore, a single host can receive transplants of cells from several donors. Donor cells and host embryos are frequently obtained using morpholino treatments, mRNA injections, transgenic, and mutant embryos. When cell transplantation is coupled with other zebrafish experimental techniques, its utility is greatly enhanced [82]. This method was used to explain the function of the tbx1 gene in the van Gogh mutant zebrafish and its possible relationship to the human DiGeorge syndrome [83]. DiGeorge syndrome is characterized by abnormalities of the thymus, aortic arch, ear, and cranial facial features [84]. Fish carrying Van Gogh mutations exhibit same phenotypic defects. To determine if the mutation that occurred during the development of the cranial face was cell autonomous. Filled van Gogh mutants with wild-type cells and studied the cartilage development in the pharynx. It has been shown that cartilage formation can be repaired by genetically modified tissues, suggesting that craniofacial abnormalities are not cell-autonomous. According to the authors, van Gogh mutants had poorer endodermal-to-developing bronchial arches signalling. Moreover, they noticed that tbx1 operates independently of cells during ear development since wild-type cells quickly integrated into the semi-circular canal to correct the ear defect [85]. Thus, using chimeric zebrafish testing led to a better understanding of the different and potential developmental processes of DiGeorge syndrome. Another technique for cell transplantation on zebrafish is called xenotransplantation, which involves inserting tumor cells into embryonic or juvenile fish [86, 87]. Mammalian tumor cells have the capacity to multiply, disseminate, and initiate angiogenesis in immunosuppressed immature fish or in zebrafish embryos prior to the complete development of the immune system. This technique can be used with transgenic cell tagging to observe the interactions between cancer cells and their in vivo surroundings. Furthermore, chemical or mutagenic screens can be used to identify genes or compounds that modify angiogenesis or tumor metastasis [88, 89]. Recently, Casper, an adult translucent zebrafish line, was described. This line is doubly homozygous due to the nacre and roy mutations, which results in the fish losing its melanocytes and iridophores and maturing into an adult with visible internal organs. High-resolution in vivo imaging techniques can be applied to these fish to study angiogenesis, metastasis, and growth in tumor transplant recipients [90]. Because it can observe interior organs in an adult organism, this fish will also be a powerful instrument for the comprehensive study of physiologic processes under both normal and pathologic conditions.

Zebrafish Cancer models:

Many cancer models based on zebrafish have been developed. We will only discuss tabular tumour models in zebrafish here because to space constraints. These are summarized in Tables 7 and 8. Every model is included in Table 7 categorized by the type of cancer and the damaged organ. Tissue-specific or ubiquitous promoter-driven oncogenes are expressed by the vast majority of transgenic lines. The genetic mutants indicated as TILLING (targeting induced local lesions in genomes) mutants or by forward genetic screens that have demonstrated the role of genes as tumor suppressors and that have been further examined to reveal their mechanism of action are listed in Table 8. Excellent recent reviews describe all of these models in detail, and they also include alternative approaches such as Xeno transplanting cancer cells into recipients that are zebrafish and using zebrafish embryos to analyse the role of oncogenes and the

biochemical signals that are activated in different types of cancer [91, 92]. The bias in the selection of tissue-specific promoters used to generate the transgenic lines is indicative of the interest of the zebrafish labs participating in the first generation of cancer models [93]. The ability of oncogenes to alter zebrafish cells across species was demonstrated by the use of human oncogenes, often in combination with fluorescent reporters to facilitate the separation and in vivo imaging of cancer cells, as well as the tracking of tumor initiation and progression [94].

Table 7: Zebrafish cancer model

Organ/ System Cancer type	Strategy	Onset (months)	Main advantages	Reference
T-ALL	c-Myc conditional	4	Delayed onset allows propagation of line	[95]
T-ALL	c-Myc transgenic	2	childhood leukaemia (CD10+B-ALL) Highly penetrate	[96]
CA exocrine pancreas	KRASV12 transgenic	6	ptf1 a promoter. Similar to human disease	[97]
Testicular cancer	ENU mutagenesis	7	Highly penetrant. Susceptibility gene still unknown	[98]
Melanoma	HRASV12 transgenic	6	mitfa promoter – late onset	[99]

Table 8: Cancer predisposition mutants

Responsible protein	Type of mutation	Type of cancer	Reference
p53	TILLING mutant	Yes, MPNST	[100]
p53	ENU mutant	Yes, sarcoma	[101]
Ribosomal proteins	Insertional mutagenesis	Yes, MPNST	[102]
Genomic stability genes	mutagenesis ENU	Yes, papilloma and others	[103]

Conclusion:

This author's aim is to outline the zebrafish model's applicability and validity in relation to a deeper comprehension of the physicochemical characteristics of zebrafish. The zebrafish model has been shown to be a viable alternative for studying the human condition based on reported research findings. In addition to its roots in genetics and developmental biology, zebrafish research is advancing a wide range of fields, such as neuroscience, behavior, memory, and cognition. Furthermore, scientists are always reproducing and verifying earlier results and developing paradigms that are similar to those reported in the literature on humans and rodents. Because the genomes of zebrafish have been sequenced and they can produce

genetic mutant models, our understanding of the genetics behind many human disease states and behaviors can be expanded. Model species like zebrafish will be used in biomedical research in the future to gain a deeper understanding of human biology. We only need to look through the vast body of rodent research to realize the options that could be available to us as zebrafish researchers. Zebrafish will remain popular as a practical and helpful model for evaluating the neurobiological model in the future. With the help of contemporary technological advancements, the zebrafish system can be used to generate new drugs, identify targets, validate existing ones, and develop them more quickly than mammalian models.
The zebrafish has established itself as a valuable animal model in biomedical research, offering a unique platform for the study of human diseases, the discovery of novel therapeutic targets, and the development of innovative treatments.

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Conflicts of Interest
The authors declare no conflict of interest

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Declaration of Competing Interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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