EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 7
Issue: 6
262-272
Year: 2020

A REVIEW ARTICLE ON "ZEBRAFISH MODELS FOR ALZHEIMER'S DISEASE"

[1]*Aamir Khan Yousuf Khan and [2]Aqsa Amtul

[1]Assistant Professor, Deccan School of Pharmacy,
[2]Student, Deccan School of Pharmacy,
Department of Pharmacology, Deccan School of Pharmacy, Dar-us-salam, Aghapura, Hyderabad 500001, Telangana, India.

***Corresponding Author: Aamir Khan Yousuf Khan**
Assistant Professor, Deccan School of Pharmacy, Department of Pharmacology, Deccan School of Pharmacy, Dar-us-salam, Aghapura, Hyderabad 500001, Telangana, India.

Article Received on 01/04/2020 **Article Revised on 22/04/2020** **Article Accepted on 12/05/2020**

ABSTRACT
Zebrafish is emerging as a valuable model for AD research and the discovery of neurodegenerative drugs. The major genes involved in human AD have homologous counterparts in zebrafish and retain function. The basic brain structure of the zebrafish is also conserved compared to the mammalian brain. An AD model was recently established by administering okadaic acid to the zebrafish and these models have been used to test the efficacy of new drugs In this review, we gave an overview of the how the zebrafish is emerging as the valuable model to study the various aspect of the AD as these fishes possess the genes orthologous to that of the humans and these models helps us to revealed the unique characteristics that were difficult using the classical rodents models. There have been various factors that allow us to model AD in zebrafish and these factors include their high degree of resemblance in-terms of neurology (neuro-anatomy, neuro-physiology), emotional and social behavioral patterns. These models have been successfully utilized to stimulate the patho-physiology of the AD and other neuro-degenerative disease like Tuopathy. Using several different models, including zebrafish, we can leverage each one's unique characteristics to reveal the molecular basis of this disease. Because of the ease of genetic malleability in zebrafish, it is easy to model Familial type of AD in them. However, in this article we would like to emphasize the need of further research and understanding of zebrafish neurology so as to aid the modelling of Sporadic type of AD.

KEYWORDS: Alzheimer's disease, dementia, neuro-degeneration, zebrafish, gamma secretase, amyloid beta-protein precursor, MAPT, presenilin, neuro-fibrillary tangles, FAD, SAD, cognitive and non-cognitive, cholinergic hypothesis, Aβ-plagues, fronto-temporal dementia, tuopathies, RFP, GFP, Morpholinos, co-orthologs, hyper-phosphorylated, autophagy, SORL1, okadaic acid, dpf, hpf, angiogenic sprouting, hypocretin/orexin neurons, GABAnergic, glutaminergic.

I – BREIF NOTE ON ALZHEIMER'S DISEASE
Alzheimer's disease (AD) can defined as the progressive neurodegenerative disease which is the most pervasive cause of dementia worldwide affecting about 47 million people worldwide.[1] The number of cases of Alzheimer's is expected to reach 82 million by 2030 and 150 million by 2050 worldwide out of which Asia-pacific is expected to contribute to about 71 million cases of AD. The net cost of AD is calculated to be $183 billion which can rise to $1.1 trillion by the end of 2050.[2] The two major pathological indications of AD are Aβ-plagues and neurofibrillary tangles.[3]

Alzheimer's disease can be predominantly classified into two types. That is, late onset AD (LOAD, occurring in people with age >65 years) and early onset AD (EOAD, occurring in people with age <65 years). While LOAD contribute to the majority of AD cases (95%) which is associated with many risk factors.[4]

Etiology of AD involve several factors such as physiological reason[5], genetic reasons[6], age, lower intelligence, small head size, and history of head trauma; female gender may confer additional risks. Susceptibility genes do not cause the disease by themselves but, in combination with other genes or epigenetic factors, modulate the age of onset and increase the probability of developing AD.[7]

Symptoms of AD may be of cognitive and non-cognitive type.[8] patho-physiology can be explained by various hypothesis such as Aβ- hypothesis[9], cholinergic hypothesis[10], tangle and calcium hypothesis[11,12] and oxidative stress hypothesis.[13]

ZEBRAFISH MODELS FOR ALZHEIMER'S DISEASSE

II-DETAIL ABOUT ZEBRAFISH AND MODELLING OF AD IN THEM

Zebrafish is a small, hardy, freshwater fish native to India and is often kept in home aquariums. They were originally used as model organisms for the study of vertebrate development. However, over the past decade, the zebrafish model has been increasingly used to study a wide variety of human diseases.[14] A zebrafish has a number of features that make it a versatile animal model. Although they lack the advanced cognitive behaviors evident in rodent models, their transparent embryos, their rapid development of the uterus and their high reproductive capacity (over 100 reproductive embryos) offer clear advantages over mammalian models. Furthermore, multiple genes can be manipulated simply and effectively in zebrafish at physiologically relevant levels,[15] which currently cannot be achieved in rodent models. Zebrafish embryos are particularly manipulable due to their large size, immediate availability and ability to exploit developmental changes for the analysis of particular genetic activities.[116] Therefore, zebrafish embryo scanning often presents a Happy vertebrate system in which examine the molecular and cellular functions of the genes involved in AD.

Zebrafish have genes that are orthologous to human genes and are believed to play an essential role in AD. The psen[117] and psen2[18] genes are human PSEN1 and PSEN2 orthologs respectively, while the appa and appb genes are "co-orthologs" of APP.[19] Zebrafish contain orthologous genes for the components of the gamma-secretase complex, PSENEN,[20.21] NCTN[22] and APH1b.[20] Although β-secretase orthologs (BACE1 and BACE2) have recently been identified in zebrafish: bace[23] and bace2.[24] The human tau protein (MAPT) gene associated with microtubules encodes the tau protein and our laboratory has identified the co-orthologs of this gene in zebrafish, mapta and maptb.[25] Zebrafish also have APOE co-orthologs apoe-a and apoe-b.[26.27] Genes that result from duplication can have overlapping functions.

Zebrafish is an advantageous model for genetic and molecular studies. Zebrafish embryos are genetically malleable by injection of antisense oligonucleotides, mRNAs, transgenes and more recently by genomic engineering systems.[28-30] Morpholinos are designed to bind to particular sites in the transcripts of a gene of interest. The binding of a morpholino can block the translation of the mRNA (knockdown) or interfere with the correct junction of the exons.[31,16,32]

Injection of sensory mRNA may allow overexpression of a particular gene of interest. The effects of morpholino and mRNA injection generally persist only during embryogenesis (2-3 days after fertilization). The transgenic zebrafish can be generated using efficient vectors such as the Tol2,[33] transposase system to insert genes under the control of specific tissue promoters.

III-ADVANTAGES OF ZEBRAFISH AS A MODEL FOR AD

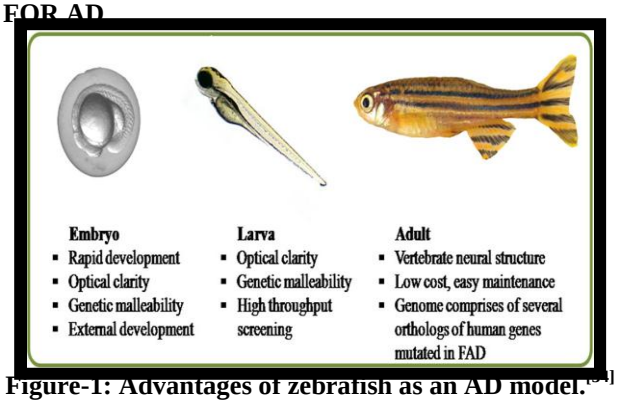

Figure-1: Advantages of zebrafish as an AD model.[34]

Neuroanatomical similarity: Recent studies have shown a high degree of homology between the human and zebrafish brain structure and in-terms of neuroanatomy and neuro-chemistry.[35-38] The medial, dorsal and lateral pallium of the zebrafish are homologous to the amygdala,[39] Iso-cortex[40] and hippocampus[41,42] in other vertebrates respectively. The zebrafish brain consists of the forebrain, mid brain and hind brain, (diencephalon, telencephalon and cerebellum). In homology to the human brain, zebrafish also possess glutamate as a excitatory and GABA as a inhibitory neurotransmitter[43] along with the acetyl-cholinergic receptor.[44] Zebrafish also possess other neurotransmitters like serotonin, histamine, dopamine and acetylcholine[45] including the enzyme for their synthesis and metabolism.[46,47] This homology even exist at the cellular level as they also possess brain cells like astrocytes,[48] microglia,[49] oligodendrocytes,[50] cerebellar Purkinje cells,[51] myelin[52] and motor neurons.[53] Further studies on adult zebrafish in this aspect shows that the process of neural differentiation and development of spinal network resembles with that of humans.[54-56]

Behavioral similarity: Scientist have utilized zebrafish as a behavioral model to study various aspects of human psychology and physiology such as feeding,[57-58] learning,[59] hearing,[61] vision,[62] touch[63-65] and emotions like fear,[66-68] pain,[69] helplessness,[70] courtship,[71] social interactions,[65,72,73] anxiety[74] and decision making.[75] further studies have reported that there exist a similarity between human and larval zebrafish in-terms of expressions and projects of hypocretin/orexin neurons.[76] making use of this high degree of similarity between zebrafish and humans, scientist also make use of circadian rhythms to understand the mechanism that regulate the sleep cycle in humans.[76-79] This works conclude that resting phase of zebrafish and the sleeping behavior of mammals have significant similarities. Furthermore, they reported that the zebrafish exerts a homeostatic control over the

resting behavior that is regulated by the circadian rhythm, characteristics similar to those of mammals.[80]

Pathophysiological resemblance: Many zebrafish models have been demonstrated by keeping Aβ- theory as center for the pathophysiology of AD. A group of scientist have suggested that a large number of Aβ monomer can stimulate the angiogenic sprouting in the hindbrain of the zebrafish.[80] However another group of scientist shows that the larvae shows cerebrovascular defects owing to the APP and Aβ deficiency. These peculiarities can be overcome by treating the embryos with $A\beta_{1-42}$ and those larvae shows no response when they were treated with p3.[81] This discovery unveiled the function of Aβ in promoting cerebrovascular growth.[82]

Besides Aβ, the scientist also stressed on developing tuopathies models of zebrafish.[83] An alteration of the cytoskeleton occurred in the expression of frontotemporal dementia with parkinsonism linked to chromosome 17, a mutant form of human Tau in the zebrafish neurons, which resembled the neurofibrillary tangles observed in AD.[84,85]

Even though the elimination of exon2 from enolase transgenic zebrafish models and its substitution with the cDNA coding for the four-repeated isoforms of human protein tau results in overexpression of protein tau in zebrafish brain by eight times when compared to that of a normal human brain. This overexpressed tau protein in zebrafish resembles with the NTFs in AD effected human and this fact exemplify the use of zebrafish models for studying AD.[86]

IV-BEHAVIORAL MODELS OF ALZHEIMER'S DISEASE

Introduction: The zebrafish locomotor activity is demonstrated in-terms of Distance Moved (DM) per unit time. These studies were conducted in both Tupfel long fin (TL) and Wild type parenteral (AB) strains of zebrafish which include studying the effects of various lightning conditions on various age group. Different age groups of both the strains (TL and AB) were exposed to various doses of ethanol and the effects of this exposure on DM of 6-days old larvae were analyzed. The other parameters like Duration of Movements (DoM) per unit time and the Velocity of Movements (DoM) were also studied. These three parameters were correlated to check their relevance in toxicity studies.

Method: zebrafish motor activity were assayed using a viewpoint behavior recording system. It also include the use of movement tracking and analysis program such as Zebrabox, Viewpoint, France that has a tracking rate of 25 testing samples per second. This set-up or apparatus consists of a chamber containing circulating water fitted with a camera above this chamber and it also has a temperature sensor. The chamber contains a microtiter plate which is irradiated with infrared or white light using a software. The larvae was then individually transferred into the squared 96-wells of the microtiter plates using a plastic pipette in a volume of ±500 µl. the applied lightning conditions was as follows: 15 min of darkness (i.e. infrared light), 15 min of bright light and a final 15-minute period of darkness (further called 'sessions': DarkI, Light, DarkII). The intensity of the light was measured using a voltcraft MS-1300 Lux meter (Voltcraft, Hirschau, Germany). The intensity of the light in the self-regulating aquaria in the laboratory housing was ±350 lx. The intensity of light in the locomotor apparatus was measured using a spherical sensor which was placed on the transparent bottom of the chamber and chamber was closed with the lid. The light were turned on with the same intensity as that used during the experiment and the resultant light intensity was measured to be 700lx and the infrared light intensity was resulted in a measured intensity of 0 lx.

All these studies and assays were carried out during afternoon that is before 2 PM in-order to ensure balanced activity of the animals used and the temperature of the water was kept constant throughout the experiment at 28^0C. Before taking the measurements, the larvae were made to adapt to the environment for a time period of 15-minutes of darkness.

The larvae of both the TL and AB strains of ages 5, 6 or 7 days were chosen for conducting the studies in group of 25-larvae each. The larvae were placed over two plates in random fashion in equal numbers and both the plates were tested subsequently. All the six groups were tested on the same day. The same protocol was followed to study the effects of ethanol or any drug that have a observable effect on the locomotor activity both in humans and mammals.

For this test, 6-day old larvae of both the strains (TL and AB, n=19/group) were used. The concentrations such as 0, 0.5, 1, 2 or 4% v/v of 100% pure ethanol were dissolved in aquarium water. These plates were analyzed consecutively. This ethanol treatment were given 30-minutes before the start of motor activity assay and were continued till the end of the assay.

Table-1 Summary of the result of studies conducted on locomotor activity.[87]

parameter	strains	Age (dfd)	Dark-I		Light		Dark-II	
			Mean[2]	SEM	Mean[2]	SEM	Mean[2]	SEM
Distance Moved (mm/min)	AB	5	152.4	4.08	27.6	2.49	119.2	5.21
		6	125.8	3.21	43.5	2.14	116.7	4.14
		7	96.1	3.23	70.0	2.81	117.4	4.24
	TL	5	104.6	4.17	24.8	1.98	76.3	4.04
		6	98.1	3.81	43.3	2.47	90.1	4.01
		7	132.5	3.57	78.1	2.89	127.6	4.20
Duration Of move-Ment (s/min)	AB	5	25.6	4.08	6.8	2.49	20.3	5.21
		6	31.0	3.21	15.4	2.14	29.1	4.14
		7	20.9	3.23	17.6	2.18	24.5	4.24
	TL	5	20.5	4.17	8.4	1.98	18.9	4.04
		6	19.8	3.81	11.5	2.47	19.1	4.01
		7	25.3	3.57	18.4	2.89	25.4	4.20
Velocity of move-ment (mm/s)	AB	5	5.79	4.08	3.81	2.49	5.29	5.21
		6	4.36	3.21	3.17	2.14	4.13	4.14
		7	4.41	3.23	3.93	2.81	4.46	4.24
	TL	5	5.01	4.17	3.35	1.98	4.16	4.04
		6	4.65	3.81	3.70	2.47	4.23	4.01
		7	4.98	3.57	4.07	2.89	4.60	4.20

Evaluation parameter: Zebrabox software were used to analyzed the tracks for all animals as Distance Moved (DM (mm)) per unit time (1-minute or 5-minute interval per 15 minute session) to determine the locomotor activity. Duration of movement were expressed at 1-minute interval. Mean of velocity of movement (mm/s) was calculated using a window excel sheet were expressed in 1-minute interval.

CONCLUSION

AB strain: Based on the results assimilated in the above table, statistical analysis were performed for the effects studied in wild-type AB larvae, which shows that the differences over time within age group were observable than that between the age groups. Nevertheless, the relation between the sessions and the age of the zebrafish was observable. Actually in the light session the Distance Moved is significantly different than the Distance Moved in both the Dark-I and Dark-II and even the values of the Distance Moved in Dark-I and Dark-II shows a significant difference. The activity of the AB 5 dpf was highest in the dark period (DarkI>DarkII) and lowest in the light period when compared to AB 6 dpf and 7 dpf. However, the differences between the age group were not significant.

TL strain: It is confirmed by the post-hoc comparison the differences in the TL- wild type are all similar to that observed in AB-wild type larvae except that in TL-wild type the level of significance is reached in all session and group comparisons.[87]

V-ASSAYS FOR AD-RELEVANT CELLULAR PATHWAYS AND PROCESSES IN ZEBRA FISH

1. γ-Secretase models of zebrafish

Introduction: There are a number of cellular pathways and processes that are aberrant in AD. The zebra fish are a useful system for investigating molecular events such as γ-secretase activity and autophagy that have been implicated in AD pathogenesis. There are over 70-proteins that are known to be substrates of γ-secretase activity.[88] In zebrafish, γ-secretase activity was initially analyzed by observing changes in the expression of genes known to be downstream targets of Notch Signaling such as hairy-related 6[89,21,90] and neurogenin.[121]

Method: Recently, the first assay to assess directly γ-secretase cleavage activity was developed by Wilson and lardelli.[91] α- and β-secretase cleavage of Appa provide substrates for subsequent γ-secretase cleavage. Unfortunately these cleaved forms of Appa cannot be detected in zebrafish embryos prior to 48hpf which currently makes monitoring endogenous Appa cleavage in manipulated zebrafish embryos difficult. Therefore, a fragment of Appa equivalent to the membrane-embedded remnant of APP following β-secretase cleavage as fused to GFP and expressed transiently in zebrafish embryos by the use of Tol2 vector trans-genesis system. This construct is co-expressed with a set ratio of free GFP (for signal normalization).

Evaluation parameter (Appa: GFP/free GFP ratio): Western immunoblotting is then used to assess the ratio of the Appa: GFP/free GFP (the γ-secretase cleavage product itself is too unstable to be observed). Once an Appa :GFP/free GFP ratio is determined for a protein sample from a pool of manipulated embryos (e.g. drug treatment, morpholino or mRNA injection) it can then be compared to control embryos to determine how that particular manipulation is affecting γ-secretase cleavage activity.

2. PROTEIN DEGRADATION PATHWAYS MODEL OF ZEBRAFISH

Introduction: Excess or aberrant cellular proteins can be degraded by the ubiquitin-proteasome system(UPS). Since protein aggregation is implicated in many neuro-degenerative diseases,[92] it is unsurprising that problems with UPS function have been implicated in neuro-degenerative disease such as AD[93-95] and Parkinson's disease.[96] Zebrafish have been used to investigate UPS function in Parkinson's disease;[97] however, no analyses in zebrafish have yet examined the UPS with respect to AD. Autophagy is an important mechanism required for the degradation of dysfunctional and unwanted cellular components (including incorrectly folded and aggregated proteins) through the actions of lysosomes. Indeed, autophagy has been identified as a pathway for the degradation of accumulated Aβ peptides.[98] Recently, the Presenilin proteins were suggested to have a major role in autophagy with FAD mutations in human PSEN1 inhibiting this function.[99] These authors presented evidence showing that PSEN1 acts as a chaperone in the ER for a transmembrane protein required for acidification of the lysosomes, the v-ATPase V0a1subunit;[99] however, other reports have disputed this finding.[100,101] They also demonstrated that this function of PSEN1 is dependent on the full-length PSEN1 holo-protein rather than the endo-proteolysed form that is active in the γ-secretase complex. Furthermore, a γ-secretase inhibitor and loss of another γ-secretase complex component (NCT) had no effect on autophagy[99] suggesting that this function of PSEN1is independent from its function in γ-secretase complexes.

Method: Autophagy can be analyzed in zebrafish by observing induction of the LC3II protein by western-immunoblotting using a human antibody against LC3 that cross reacts with zebrafish Lc3.[102,103] Transgenic zebrafish have also been developed that express GFP fused to Lc3.[102] As Presenilin protein expression can easily be manipulated in the zebrafish these autophagy assay are a useful tool for further investigation of the involvement of the Presenilins in autophagy.

Evaluation parameter: Degradation of accumulated Aβ peptides.

VI – USING THE ZEBRAFISH TO INVESTIGATE OTHER ASPECTS OF ALZHEIMER'S DISEASE ETIOLOGY

3. HYPOXIA MODEL OF ZEBRAFISH

Introduction: There is accumulating evidence suggesting that hypoxia is an important initiating factor in the pathogenesis of AD. Under hypoxic conditions the electron transport chain in the mitochondria increases free radical production that leads to increased oxidative stress.[104] Biomarkers of hypoxia can differentiate between people with mild cognitive impairment that progress to AD and those who do not.[105] The risk factors for cardiovascular disease and AD are similar[106] and it is anticipated that vasculature problems would affect oxygenation of the brain. Interestingly, Aβ levels in serum have been shown to be elevated after cardiac arrest.[107] Zebrafish are an advantageous system for analysis of the effects of hypoxia on various biological functions.

Method: Zebrafish embryos and adults can be exposed to real hypoxia by depleting their water environment of oxygen or to chemical mimicry of hypoxia through, e.g.,sodium azide treatment.[108] Similarly to what is observed in humans,[109-111] hypoxia upregulates psen1, psen2, appa, appb, and bace1 in zebrafish adult brain and larvae.[23] This suggests that Aβ is produced as a protective response to hypoxia in both human and zebrafish cells – a response conserved over 450 Mya of evolutionary time.

Note: however that while all the enzymes required to cleave Aβ from Appa and/or Appb are present in zebrafish, the existence of Aβ itself has not yet been directly demonstrated (e.g through immune-blotting or mass spectrometry). The study by Moussavi Nik et al.[23] also demonstrated that, unlike in mammals, F2-isoprostanes are not a good marker of oxidative stress in zebrafish and that the upregulation of catalase gene expression can be a better alternative marker for demonstration of oxidative stress in zebrafish.[112,113]

Evaluation parameter: Hypoxia upregulates psen1, psen2, appa, appb, and bace1 in zebrafish adult brain and larvae (Moussavi Nik et al.,2012). This suggests that Aβ is produced as a protective response to hypoxia in both human and zebrafish cells.

4. APOE MODEL OF ZEBRAFISH

The APOE ε4 allele has been identified as the main genetic risk factor for SAD. APOE is important for clearance of amyloid-beta from the brain,[114] while the AD risk associated ε4 allele has been shown to impair the clearance of Aβ[115] and, more recently, to affect the integrity of the blood–brain barrier.[116] There has been little research investigating APOE function in zebrafish. Expression studies of apoe-a[117] and apoe-b[118] revealed expression in the developing retina and yolk syncytial layer. Furthermore, apoe-b has also been observed in microglial cells,[119] developing fins and epidermis,[120,121] re-generating fin tissue,[120] macrophages,[122] liver, intestine, and ovary.[123]

Measured parameter: Impairment in the clearance of amyloid-β from the brain and integrity of BBB.

5. MAPT MODEL OF ZEBRAFISH

Introduction: The MAPT is the main component of the NFTs found in AD brains. Various dysfunctions of the tau protein are found in other neuro-degenerative disorders such as fronto-temporal dementia (FTD), cortico basal degeneration and progressive supra-nuclear palsy.[124] Diseases with tau like pathology are collectively termed "tauopathies" are view by Bai and

Burton (2011)[125] discussed how the zebrafish has been used to investigate these diseases. A number of MAPT protein isoforms exist as a result of alternative splicing of MAPT transcripts. These isoforms can be classified into two groups, 3R or 4R, depending on the number of tubulin-binding motifs. It appears that an overall one-to-one ratio of 3R to 4R transcripts is required for normal functioning of the MAPT protein in the brain.[125] In most of the tauopathies this ratio is found to be changed[126] and altered, splicing of MAPT is also suggested to occur in AD brains.[127]

Method: Transgenic zebrafish expressing human MAPT were generated and investigated prior to identification of the zebrafish ortholog (s) of MAPT. In these studies human MAPT was specifically expressed in zebrafish CNS neurons.[128,129] Bai et al. used the promoter of the enolase2 gene to drive the expression of MAPT 4R in zebrafish neurons at approximately eight fold higher levels than what is observed in human brain. This resulted in accumulations of tau-protein (resembling NFTs) in the zebrafish brain. In the study by Paquet et al., the HuC promoter was employed to drive expression of a Gal4:VP16 fusion protein in neurons. This protein was then bound to UAS sites in a bi-directional promoter transcribing the DsRed fluorescent marker protein gene and a mutant form of human MAPT associated with FTD, TAU-P301L. The transgenic zebrafish larvae showed biochemical changes consistent with those observed in human tauopathies. However, it should be noted that no comparisons of phenotype were made between the non-mutant and mutant forms of human MAPT in the zebrafish. Furthermore, the expression levels of the transgenes relative to the endogenous zebrafish mapt genes were not assessed in these studies. Despite these limitations these transgenic zebrafish models provide a useful system to investigate whether chemical inhibitors can modulate the observed tauopathy-associated changes.

The zebrafish "co-orthologs" of the human MAPT gene, mapt-a and mapt-b have similar but not completely overlapping patterns of expression in developing embryos.[130] They are both predominantly expressed in the developing CNS while only mapt-b has strong expression in the trigeminal ganglion and dorsal sensory neurons of the spinal cord. Mapt-a is spliced into 4R–6R isoforms while mapt-b is spliced mainly into 3R isoforms. Manipulation of the expression of the zebrafish mapt isoforms may therefore be advantageous for understanding the function of 3R and 4R MAPT and the role(s) the 3R:4R ratio plays in pathogenesis.

Evaluation parameter: 3R: 4R ratio is measured. An overall one-to-one ratio of 3R to 4R transcripts is required for normal functioning of the MAPT protein in the brain. Any abnormalities in these ratios shows alteration in the normal physiology of brain and the formation of NFTs.

6. AMYLOID PRECURSOR PROTEIN MODELS OF ZEBRAFISH

Introduction: The zebrafish *APP* "co-orthologs," *appa* and *appb* have widespread and overlapping expression from mid-gastrulation in the developing embryo(Musaetal.,2001). At 24hpf both genes are expressed in the developing forebrain and other tissues with only *appb* expressed in the spinal cord (Musaetal.,2001).

Methods: In a zebrafish study by Lee and Colea, section of *appb* regulatory sequence was fused to GFP (LeeandCole,2007). Dissimilar to other expression studies, they observed expression of *appb* in the developing vasculature. More recently, Liao et al.(2012) isolated transposon gene trap integrations that contained RFP in the *appa* gene and in the closely related a*myloid beta precursor-like protein2gene(aplp2).*

The gene traps caused fusions to RFP of the extra-cellular domains of both of the encoded proteins. The fusion proteins of these genes were accumulated in the vasculature. However, they could not detect the transcripts of these genes in the endothelial cells of the vasculature. Instead, transcripts were detected in neuronal cells. This suggests that these proteins are synthesized in neuronal cells and then accumulate in the vasculature. Translation blocking morpholinos have also been employed to investigate the function of the Appa and Appb proteins (Joshi et al.,2009). Inhibition of Appa had little effect on the developing embryo while Appb translation inhibition resulted in defective convergent extension cellular movements and reduced body length.
These defects could be rescued by injecting Appb deficient embryos with mRNA coding for human APP. The rescue by human APP was more effective than injection of an mRNA encoding a human *APP* FAD mutant (the APP Swedish double mutation *K595N* and *M596L*). Loss of Appb activity has also been shown to cause defective neural development (Song and Pimplikar,2012) including defective axonal out-growth patterning and synapse formation (Abramssonetal.,2013). In the study by Song and Pimplikar, only full-length human APP but not truncated forms could rescue the neuronal defects, revealing that both intracellular and extracellular domains of human APP are required for normal function (Song and Pimplikar, 2012). These studies demonstrate that zebrafish embryos can be exploited for the analysis of different mutant forms of human *APP*.[131]

Evaluation parameter: Detection or analysis of various different mutant forms of APP as their deposition in brain is one of the causative factor for AD pathology.

Table-2 Assay of various AD relevant cellular pathways in zebrafish.[131,104-130]

Processes	Measured parameter.
γ- secretase activity	APPa : GFP/free GFP
Protein degradation pathway	Degradation of accumulated Aβ peptide
Hypoxia	Upregulation of psen1, psen2, appa, appb, and bace1
APOE	Impairment in the clearance of amyloid-β from the brain
MAPT	3R: 4R ratio is measured.
APP	Detection of various mutant forms of APP

VII-THE FUTURE OF MODELING ALZHEIMER'S DISEASE IN ZEBRAFISH

Zebrafish is rapidly emerging as an attractive model for AD research. They are an ideal model for drug tests before clinical trials on rodents. However, there are still aspects of this model that require better understanding. In order for the zebrafish system to be used to model aspects of AD pathobiology, we must better understand the structure and function of the zebrafish brain and also gain a deeper understanding of the physiology of the adult brain of the zebrafish. The work done so far has revealed that the brain of the zebrafish has a reasonable level of conservation of the basic structure compared to mammals, as well as neuroanatomical and neurochemical pathways similar to those that play a role in human diseases.[132] We have revealed various aspects of genetic biology of presenilin using zebrafish that would otherwise be difficult to observe / analyze in other models. However, to effectively analyze the future transgenic and mutant models of AD zebrafish, we must strengthen our understanding of the functions in zebrafish of some of the orthologs of key genes involved in the pathogenesis of human AD such as MAPT and APOE.

It is questionable whether zebrafish can be used to model a late-onset disease such as AD, since zebrafish has a profound regenerative capacity and this should influence the development of neurodegenerative phenotypes. The neurogens in the brain of the adult zebrafish are much more abundant than those observed in mammals. As a result, it makes it difficult to analyze neuronal loss. Despite these limitations, the availability of cents and the feasibility of using genome modification technologies represent an exciting opportunity to develop zebrafish genetic models for neurodegenerative diseases such as AD. ZFN, TALEN and CRISP / R have been validated for use in zebrafish [revised by Hwang et al., 2013; SchmidandHaass, 2013) and it is inevitable that FAD mutations are introduced into the orthologists of the zebrafish FAD gene.

Animal models are a useful tool for studying the causes of human disease pathologies. Obviously, such models can never reflect the complete pathology seen in human cases. The complexity of the human brain makes AD a particularly difficult disease to model in animals. However, by using several different models, including zebrafish, we can take advantage of the unique characteristics of each to reveal the molecular basis of this disease.

REFERENCES

1. Cummings, J. L. Alzheimer's disease. N. Engl. J. Med. 351: 56–67 (2004).
2. Stefanacci, R. G. The costs of Alzheimer's disease and the value of effective therapies. Am. J. Manag. Care 17(Suppl 13): S356–S362 (2011).
3. Serrano-Pozo et al., Neuropathological alterations in Alzheimer disease. Cold Spring Harb. Perspect. Med. 1: a006189 (2011).
4. I.J. Martins et al., Apolipoprotein E, cholesterol metabolism, diabetes, and the convergence of risk factors for Alzheimer's disease and cardiovascular disease, Mol. Psychiatry, 2006; 11: 721–736.
5. Department of Neuroscience, Osaka City University Graduate School of Medicine, Abeno-ku, Osaka, Japan. Brain and Nerve Shinkei Kenkyu no Shinpo, 01 Jul 2010; 62(7): 691-699Language:jpn PMID: 20675873 review on: Involvement of beta-amyloid in the etiology of Alzheimer's disease.
6. Corinne L. Lendon, PhD; Frank Ashall, DPhil; Alison M. Goate, DPhil -" Exploring The Etiology By Using Molecular Genetics". From the Departments of Psychiatry (Drs Lendon and Goate) and Genetics (Dr Goate), Washington University School of Medicine, and Saint Louis University School of Medicine (Dr Ashall), St Louis, Mo. JAMA. 1997; 277(10): 825-831. doi:10.1001/jama.1997.03540340059034 march-1997
7. Jeffrey L. Cummings, Harry V. Vinters, Gregory M. Cole, Zaven S. Khachaturian. "Alzheimer's disease-Etiologies, pathophysiology, cognitive reserve, and treatment opportunities". First published July 1, 1998,DOI:https://doi.org/10.1212/WNL.51.1_Suppl_1.S2.
8. Joseph T. DiPiro, Pharmacotherapy: A Pathophysiologic Approach, Sixth Edition, 2005.
9. Hardy, J. A. & Higgins, G. A. Alzheimer's disease: the amyloid cascade hypothesis. Science 256: 184–185 (1992).
10. Francis, P. T., Palmer, A. M., Snape, M. & Wilcock, G. K. The cholinergic hypothesis of Alzheimer's disease: a review of progress. J. Neurol. Neurosurg. Psychiatry 66: 137–147 (1999).

11. Berridge, M. J. Calcium hypothesis of Alzheimer's disease. Pflug. Arch.: Eur. J. Physiol. 459: 441–449 (2010).
12. Alzheimer's Association Calcium Hypothesis. W. Calcium Hypothesis of Alzheimer's disease and brain aging: a framework for integrating new evidence into a comprehensive theory of pathogenesis. Alzheimers Dement. 13: 178–182 e117 (2017).
13. Torres LL, Quaglio NB, de Souza GT, Garcia RT, Dati LM, Peripheral oxidative stress biomarkers in mild cognitive impairment andAlzheimer's disease. J Alzheimers Dis 2011; 26: 59-68.
14. Lieschke, G. J., et al (2007).Animal models of human disease:zebrafish swim into view. *Nat.Rev.Genet.* 8: 353–367.doi:10.1038/nrg2091.
15. Newman, M., et al (2012).Robust homeostasis of Presenilin1 protein levels by transcript regulation. *Neuro sci.Lett.* 519: 14–19. doi:10.1016/j.neulet.2012.04.064.
16. Nornes, S., et al (2009).Independent and co-operative action of Psen2 with Psen1in zebrafish embryos. *Exp.CellRes.* 315: 2791–2801.doi:10.1016/j.yexcr.2009. 06.023.
17. Leimer, U., et al. (1999). Zebrafish (*Danio rerio*) presenilin promotes aberrant amyloid beta- peptide production and requires a critical aspartate residue for its function in amyloidogenesis. *Biochemistry* 38: 13602–13609.doi:10.1021/bi99 1453n.
18. 18.Groth, C., et al (2002).Identification of a second presenilin gene in zebrafish with similarity to the humanAlzheimer's disease gene presenilin2. *Dev.GenesEvol.* 212: 486–490.doi:10.1007/s00427-002-0269-5.
19. Musa, A., et al (2001).Distinct expression patterns of two Zebrafish homologues of the human APP gene during embryonic development. Dev.GenesEvol. 211: 563–567.doi:10.1007/s00427-001-0189-9.
20. Francis, R., et al.(2002). aph-1 and pen-2 are required for notch pathway signaling, gamma-secretase cleavage of betaAPP, and presenilin protein accumulation. Dev.Cell 3: 85–97. doi: 10.1016/S1534-5807(02)00189-2.
21. Campbell, W. A., et al.(2006).Zebrafish lacking Alzheimer presenilin enhancer 2 (Pen-2) demonstrate excessive p53-dependent apoptosis and neuronal loss. J. Neurochem. 96: 1423–1440.doi:10.1111/j.1471-4159.2006.03648.x.
22. Strausberg, R. L., et al.(2002).Generation and initial analysis of more than15,000 full-length human and mouse cDNA sequences. *Proc.Natl.Acad.Sci.U.S.A.* 99: 16899–16903. doi:10.1073/pnas.242603899.
23. Moussavi Nik, S. H., et al.(2012).TheBACE1-PSEN-A betaPP regulatory axis has an ancient role in response to low oxygen/oxidative stress. *J. AlzheimersDis.* 28: 515–530.doi: 10.3233/JAD-2011-110533.
24. van Bebber, F., et al (2013).Loss of Bace2 in zebrafish affects melanocyte migration and is distinct from Bace1 knock out phenotypes. J. Neurochem.127: 471–481.doi:10.1111/jnc.12198.
25. Chen, M., et al (2009).Complex splicing and neural expression of duplicated tau-genes in zebrafish embryos. *J. AlzheimersDis.* 18: 305–317. doi:10.3233/JAD-2009-1145.
26. Babin, P. J., et al (1997).Both apolipoproteinE and A-I genes are present in a non-mammalian vertebrate and are highly expressed during embryonic development. *Proc.Natl.Acad.Sci.U.S.A.* 94: 8622–8627.doi:10.1073/pnas.94. 16.8622.
27. Woods, I. G., et al.(2005). The zebrafish gene map defines ancestral vertebrate chromosomes. *GenomeRes.* 15: 1307–1314.doi:10.1101/gr.4134305.
28. Hwang, W. Y., et al.(2013).Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat.Biotechnol. 31: 227–229.doi:10.1038/nbt.2501.
29. Schmid, B., and Haass,C. (2013).Genomic editing opens new avenues for Zebrafish as a model for neurodegeneration. J. Neurochem. 127: 461–470.doi:10.1111/jnc.12460.
30. Hisano, Y., et al (2014).Genome editing using artificial site-specific nucleases in zebrafish. *Dev. Growth Differ.* 56: 26–33.doi: 10.1111/dgd.12094.
31. Draper, B. W., et al (2001).Inhibition of zebrafish fgf8 pre-mRNA splicing with morpholino oligos: a quantifiable method for gene knockdown. *Genesis* 30: 154–156.doi:10.1002/gene.1053.
32. Berger, J., et al Berger, S., Jacoby, A. S., Wilton, S. D., and Currie, P. D. (2011).Evaluation of exon-skipping strategies for Duchenne muscular dystrophy utilizing dystrophin-deficient zebrafish. *J. Cell Mol .Med.* 15: 2643–2651.doi: 10.1111/j.1582-4934.2011.01260.x.
33. Kawakami, K., et al (2000). Identification of a functional transposase of the Tol2 element,an Ac-like element from the Japanese medaka fish, and its transposition in the zebrafish germ lineage. *Proc.Natl.Acad.Sci. U.S.A.* 97: 11403–11408.doi:10.1073/pnas.97.21.11403.
34. Monnot et al,.(1999). Epidermal expression of apolipoprotein-E gene during fin and scale development and fin regeneration in zebrafish. *Dev.Dyn.* 214: 207–215. doi:10.1002/(SICI)1097-0177(199903)214:3<207::AID-AJA4>3.0. CO;2-5.
35. Panula, P. et al. (2006) Modulatory neurotransmitter systems and behavior: towards zebrafish models of neurodegenerative diseases. Zebrafish 3, 235–247.
36. Chen, Y. C., et al.(2009) Complementary developmental expression of the two tyrosine hydroxylase transcripts in zebrafish. Histochem. Cell Biol. 132: 375–381.
37. Anichtchik, O., et al (2006) Distinct structure and activity of monoamine oxidase in the brain of zebrafish (Danio rerio). J. Comp. Neurol. 498: 593–610.
38. Kawai, H., Arata, N. & Nakayasu, H.(2001) Three-dimensional distribution of astrocytes in zebrafish spinal cord. Glia 36: 406–413.

39. Peri, F. & Nusslein-Volhard, C.(2008) Live imaging of neuronal degradation by microglia reveals a role for v0-ATPase a1 in phagosomal fusion in vivo. Cell 133: 916–927.
40. Yoshida, M. &Macklin, W. B.(2005) Oligodendrocyte development and myelination in GFP-transgenic zebrafish. J. Neurosci. Res. 81: 1–8.
41. Koulen, P., et al (2000) Conservation of localization patterns of IP(3) receptor type 1 in cerebellar Purkinje cells across vertebrate species. J. Neurosci. Res. 61: 493–499.
42. Avila, R. L., et al (2007). Myelin structure and composition in zebrafish. Neurochem. Res. 32: 197–209.
43. Westerfield,M., et al (1986) Identified motoneurons and their innervation of axial muscles in the zebrafish. J. Neurosci. 6: 2267–2277.
44. Stil, A. & Drapeau, P.(2016) Neuronal labeling patterns in the spinal cord of adult transgenic Zebrafish. Dev. Neurobiol. 76: 642–660.
45. Chong, M. & Drapeau, P. (2007) Interaction between hind brain and spinal networks during the development of locomotion in zebrafish. Dev. Neurobiol. 67: 933–947.
46. Madgwick, A. et al. (2015) Neural differentiation modulates the vertebrate brain specific splicing program. PLoS. ONE 10: e0125998.
47. Gahtan, E., et al (2005) Visual prey capture in larval zebrafish is controlled by identified reticulospinal neurons downstream of the tectum. J. Neurosci. 25: 9294–9303.
48. Del Bene, F. et al. (2010) Filtering of visual information in the tectum by an identified neural circuit. Science 330: 669–673.
49. Bianco, I. H., et al (2011) Prey capture behavior evoked by simple visual stimuli in larval zebrafish. Front. Syst. Neurosci. 5: 101.
50. Valente, A., et al (2012) Ontogeny of classical and operant learning behaviors in zebrafish. Learn. Mem. 19: 170–177.
51. Gleason, M. R. et al.(2009) The transmembrane inner ear (Tmie) protein is essential for normal hearing and balance in the zebrafish. Proc. Natl Acad. Sci. USA 106: 21347–21352.
52. Emran, F. et al.(2007) OFF ganglion cells cannot drive the optokinetic reflex in zebrafish. Proc. Natl Acad. Sci. USA 104: 19126–19131.
53. Low, S. E. et al. (2010) touche Is required for touch-evoked generator potentials within vertebrate sensory neurons. J. Neurosci. 30: 9359–9367.
54. Low, S. E. et al.(2011) TRPM7 is required within zebrafish sensory neurons for the activation of touch-evoked escape behaviors. J. Neurosci. 31: 11633–11644.
55. Low, S. E. et al. (2012) Touch responsiveness in zebrafish requires voltage-gated calcium channel 2.1b. J. Neurophysiol. 108: 148–159.
56. Speedie, N. & Gerlai, R. (2008) Alarm substance induced behavioral responses in zebrafish (Danio rerio). Behav. Brain. Res. 188: 168–177.
57. Agetsuma, M. et al. (2010) The habenula is crucial for experience-dependent modification of fear responses in zebrafish. Nat. Neurosci. 13: 1354–1356.
58. Mathuru, A. S. et al.(2012) Chondroitin fragments are odorants that trigger fear behavior in fish. Curr. Biol. 22: 538–544.
59. Prober, D. A. et al. (2008) Zebrafish TRPA1 channels are required for chemo-sensation but not for thermos-sensation or mechano-sensory hair cell function. J. Neurosci. 28: 10102–10110.
60. Lee, A. et al. (2010) The habenula prevents helpless behavior in larval zebrafish. Curr. Biol. 20: 2211–2216.
61. Darrow, K. O. & Harris, W. A. Characterization and development of courtship in zebrafish, Danio rerio. Zebrafish-1, 40–45 (2004). Saleem and Kannan Cell Death Discovery (2018) 4: 45 Page 10 of 13 Official journal of the Cell De ath Differentiation Association
62. Mahabir, S., et al (2013) Maturation of shoaling in two zebrafish strains: a behavioral and neurochemical analysis. Behav. Brain. Res. 247: 1–8.
63. Qin, M., Wong, A., et al (2014) Induction of social behavior in zebrafish: live versus computer animated fish as stimuli. Zebrafish 11: 185–197.
64. Stewart, A. et al. (2012) Modeling anxiety using adult zebrafish: a conceptual review. Neuropharmacology 62: 135–143.
65. Arganda, S., et al (2012) A common rule for decision making in animal collectives across species. Proc. Natl Acad. Sci. USA 109: 20508–20513.
66. Prober, D. A., et al (2006) Hypocretin/orexin overexpression induces an insomnia-like phenotype in zebrafish. J. Neurosci. 26: 13400–13410.
67. Zhdanova, I. V. Sleep in zebrafish. Zebrafish 3: 215–226 (2006).
68. Yokogawa, T. et al.(2007) Characterization of sleep in zebrafish and insomnia in hypocretin receptor mutants. PLoS Biol. 5: e277.
69. Rihel, J. et al.(2010) Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. Science 327: 348–351.
70. Zhdanova, I. V.,(2001) Melatonin promotes sleep-like state in zebrafish. Brain Res. 903: 263–268.
71. Cameron, D. J. et al.(2002) Alzheimer's-related peptide amyloid-beta plays a conserved role in angiogenesis. PLoS. ONE 7: e39598.
72. Donnini, S. et al.(2010) A beta peptides accelerate the senescence of endothelial cells in vitro and in vivo, impairing angiogenesis. FASEB J. 24: 2385–2395.
73. Dujardin, S., (2005) Invited review: Animal models of tauopathies and their implications for research/translation into the clinic. Neuropathol. Appl. Neurobiol. 41, 59–80.
74. Bai, Q. & Burton, E. A.(2011) Zebrafish models of tauopathy, Biochim, Biophys. Acta 1812: 353–363.

75. Tomasiewicz, H. G., et al (200) Transgenic zebrafish model of neurodegeneration. J. Neurosci. Res. 70: 734–745.
76. Bai, Q., et al (2007) Generation of a transgenic zebrafish model of Tauopathy using a novel promoter element derived from the zebrafish eno2 gene.Nucleic Acids Res. 35: 6501–6516.
77. Best, J. D. et al. Non-associative learning in larval zebrafish. Neuro psycho-pharmacology 33: 1206–1215 (2008).
78. Nery, L. R. et al. Brain intraventricular injection of amyloid-beta in zebrafish embryo impairs cognition and increases tau phosphorylation, effects reversed by lithium. PLoS. ONE 9: e105862 (2014).
79. Paquet, D., Schmid, B. & Haass, C. Transgenic zebrafish as a novel animal model to study tauopathies and other neurodegenerative disorders in vivo. Neurodegener. Dis. 7: 99–102 (2010).
80. Joshi, P., Liang, J. O., DiMonte, K., Sullivan, J. & Pimplikar, S. W. Amyloid precursor protein is required for convergent-extension movements during Zebrafish development. Dev. Biol. 335: 1–11 (2009).
81. Mullan, M. et al. A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. Nat. Genet. 1: 345–347 (1992).
82. Rogaeva, E. et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat. Genet. 39: 168–177 (2007).
83. beta-peptide production and requires a critical aspartate residue for its function in amyloido-genesis. Biochemistry 38: 13602–13609 (1999).
84. Sundvik, M., Chen, Y. C. & Panula, P. Presenilin1 regulates histamine neuron development and behavior in zebrafish, danio rerio. J. Neurosci. 33: 1589–1597 (2013)
85. van Bebber, F., Hruscha, A., Willem, M., Schmid, B. & Haass, C. Loss of Bace2 in zebrafish affects melanocyte migration and is distinct from Bace1 knock out phenotypes. J. Neurochem. 127: 471–481 (2013).
86. Newman, M. et al. A zebrafish melanophore model of amyloid beta toxicity. Zebrafish 7: 155–159 (2010).
87. Celine de Esch et al. Locomotor activity assay in zebrafish larvae: Influence of age, strain and ethanol 34: 425–433 (2012).
88. Lleo, A., and Saura,C.A.(2011).Gamma-secretase substrates and their implications for drug development in Alzheimer's disease. *Curr.Top.Med.Chem.* 11: 1513– 1527. doi:10.2174/156802611795861004.
89. Bernardos, R. L., et al (2005).Notch-Delta signaling is required for spatial patterning and Muller glial differentiation in the zebrafish retina. *Dev .Biol.* 278: 381–395.doi:10.1016/j.ydbio.2004.11.018.2
90. Lleo, A., and Saura,C.A.(2011).Gamma-secretase substrates and their implications for drug development in Alzheimer's disease. *Curr.Top.Med.Chem.* 11: 1513– 1527. doi:10.2174/156802611795861004.
91. Bernardos, R. L., et al (2005).Notch-Delta signaling is required for spatial patterning and Muller glial differentiation in the zebrafish retina. *Dev .Biol.* 278: 381–395.doi:10.1016/j.ydbio.2004.11.018.
92. Arslanova, D., et al (2010).Phenotypic analysis of images of zebrafish treated withAlzheimer's gamma-secretase inhibitors. *BMC Biotechnol.* 10: 24. doi:10.1186/1472-6750- 10-24.
93. Wilson, L., and Lardelli, M. (2013). The development of an in-vivo gamma-secretase assay using zebrafish embryos. *J. Alzheimer's Dis.* 36: 521-534. doi:10.3233/JAD- 130332.
94. Ross,C.A., et al (2004).Proteinaggregationandneurodegenerative disease. *Nat.Med.* 10(Suppl.), S10–S17.doi:10.1038/nm1066.
95. West., et al (1994).Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* 344: 769–772.doi:10.1016/S0140-6736(94) 92338-8.
96. Nikitin., et al (2003a).Pathway studio–the analysis and navigation of molecular networks.*Bio-informatics* 19: 2155–2157. doi: 10.1093/bioinformatics/btg290.
97. Novichkova., et al (2003b). MedScan, a natural language Processing engine for MEDLINE abstracts. Bioinformatics 19: 1699–1706.doi:10.1093/bioinformatics/btg207.
98. Olanow,. et al (2006).Ubiquitin-proteasome system and Parkinson's disease. *Mov.Disord.* 21: 1806–1823.doi:10.1002/mds.21013.
99. Regeur., et al .(1994). No global neocortical nerve cell loss in brains from patients with senile dementia of Alzheimer's type. *Neurobiol. Aging* 15: 347–352.doi: 10.1016/0197-4580(94)90030-2.
100. Nilsson., et al. (2013). A beta secretion and plaque formation depend on autophagy. *Cell Rep.* 5: 61–69. doi:10.1016/j.celrep.2013.08.042.
101. Lee., et al.(2010). Lysosomal proteolysis and autophagy require presenilin1and are disrupted by Alzheimer related PS1 mutations. *Cell* 141: 1146–1158.doi: 10.1016/j.cell.2010.05.008.
102. Coen., et al.(2012). Lysosomal calcium homeostasis defects not proton pump defects, cause endo-lysosomal dysfunction in PSEN-deficient cells. *J. Cell Biol.* 198: 23–35. doi: 10.1083/jcb.201201076 .
103. Zhang ., et al. (2012). A role for presenilins in autophagy revisited: normal acidification of lysosomes in cells lacking PSEN1and PSEN2. *J. Neurosci.* 32: 8633–8648.doi: 10.1523/JNEUROSCI.0556-12.2012.
104. He, C., et al,. (2009). Assaying autophagic activity in transgenic GFP-Lc3 and GFP-Gabarap zebrafish embryos. *Autophagy* 5: 520–526.doi:10.4161/auto.5.4.7768.
105. Klionsky et al,. (2010). Analyzing autophagy in zebrafish. *Autophagy* 6: 642–644. doi:10.4161/auto.6.5.12092.

106. Bell et al,. (2007).The Q(o) site of the mitochondrial complex-III is required for the transduction of hypoxic signaling via reactive oxygen species production. *J. CellBiol.* 177: 1029–1036.doi:10.1083/jcb.200609074.
107. Oresic et al,. (2011). Metabolome in progression to Alzheimer's disease. *Transl. Psychiatry* 1: e57. doi:10.1038/tp.2011.55.
108. Kotze et al,. (2012). Pathology supported genetic testing and treatment of cardiovascular disease in middle age for prevention of Alzheimer's disease. *Metab. Brain Dis.* 27: 255–266.doi:10.1007/s11011-012-9296–9298.
109. Zetterberg et al,. (2011). Hypoxia due to cardiac arrest induces a time-dependent increase in serum amyloid beta levels in humans. *PLoS ONE* 6: e28263. doi: 10.1371/journal.pone.0028263.
110. Moussavi Nik et al,. (2011).The response of HMGA1 to changes in oxygen availability is evolutionarily conserved. *Exp. Cell Res*. 317: 1503–1512. doi:10.1016/j.yexcr.2011.04.004.
111. Lukiw et al,. (2001). Presenilin-2 (PS2) expression up-regulation in a model of retinopathy of prematurity and patho-angiogenesis. *Neuroreport* 12: 53–57doi: 10.1097/00001756-200101220-00019.
112. Pluta, R.(2005). Pathological opening of the blood-brain barrier to horseradish peroxidase and amyloid precursor protein following ischemia-reperfusion brain injury. *Chemotherapy* 51: 223–226.doi:10.1159/000086924.
113. Zhang, X et al,. (2010). Pathological role of hypoxia in Alzheimer's disease. *Exp.Neurol.* 223: 299–303.doi:10.1016/j.expneurol.2009.07.033.
114. Jin et al,. (2011). Cypermethrin has the potential to induce hepatic oxidative stress, DNA damage and apoptosis in adult zebrafish (*Danio rerio*). *Chemosphere* 82: 398–404.doi: 10.1016/j.chemosphere.2010.09.072.
115. Tseng et al,. (2013). Genetic Variations of GAK in two Chinese Parkinson's disease populations: a case control study. *PLoS ONE* 8: e67506. doi:10.1371/journal.pone.0067506.
116. Huang et al,. (2012). Alzheimer mechanisms and therapeutic strategies. *Cell* 148, 1204–1222.doi:10.1016/j.cell.2012.02.040.
117. Deane et al,. (2008). Apo-E isoform specified disruption of amyloid beta peptide clearance from mouse brain. *J. Clin.Invest.* 118: 4002–4013.doi:10.1172/JCI3666336663.
118. Bell et al,. (2012). Apolipoprotein-E controls cerebrovascular integrity via cyclophilinA. *Nature* 485: 512–516. doi:10.1038/nature11087.
119. Raymond et al., (2006). Molecular characterization of retinal stem cells and their niches in adult zebrafish. *BMC Dev.Biol.* 6: 36. doi:10.1186/1471-213X-6–36.
120. Pujic et al,. (2006). Reverse genetic analysis of neuro-genesis in the zebrafish retina. *Dev.Biol.* 293: 330–347. doi:10.1016/j.ydbio.2005.12.056.
121. Veth et al,. (2011). Mutations in zebrafish lrp2 result in adult-onset ocular pathogenesis that models myopia and other risk factors for glaucoma. *PLoSGenet.* 7: e1001310. doi: 10.1371/journal.pgen.1001310.
122. Tingaud-Sequeira et al., (2006). Epidermal transient down-regulation of retinol-binding protein 4and mirror expression of apo-lipoprotein Eb and estrogen receptor-2a during zebrafish fin and scale development. *Dev.Dyn.* 235: 3071–3079.doi:10.1002/dvdy. 20921.
123. Lien et al,. (2006). Gene expression analysis of zebrafish heart regeneration. *PLoSBiol.* 4: e260. doi: 10.1371/journal.pbio.0040260.
124. Levi et al., .(2012).Insight into molecular pathways of retinal metabolism, associated with vitello genesis in zebrafish. *Am. J. Physiol. Endocrinol. Metab.* 302: E626–E644.doi: 10.1152/ajpendo.00310.2011.
125. Pittman et al., (2006).Untangling the tau-gene association with neurodegenerative disorders. *Hum.Mol.Genet.* 15 (Spec No.2), R188–R195. doi:10.1093/hmg/ddl190.
126. Bai et al., (2011). Zebrafish models of tauopathy. *Biochim. Biophys. Acta* 1812: 353–363.doi:10.1016/j.bbadis.2010.09.004.
127. Goeder et al., (2006).AcenturyofAlzheimer'sdisease. *Science* 314, 777–781.doi:10.1126/science.1132814.
128. Conrad et al., (2007). Single molecule pro-filing of tau-gene expression in Alzheimer's disease. *J. Neurochem.* 103: 1228–1236.doi:10.1111/j.1471-4159.2007.04857.x.
129. Bai et al., (2007).Generation of a transgenic zebrafish model of Tauopathy using an ovel promoter element derived from the zebrafish eno2 gene. *NucleicAcidsRes.* 35: 6501–6516.doi: 10.1093/nar/gkm608.
130. Paquet et al., (2009). A zebrafish model of tauopathy allows in-vivo imaging of neuronal cell death and drug evaluation. *J. Clin. Invest.* 119: 1382–1395.doi:10.1172/JCI37537.
131. Chen, M et al., (2009).Complex splicing and neural expression of duplicated tau genes in zebrafish embryos. *J. Alzheimer's Dis.* 18: 305–317. doi:10.3233/JAD-2009-1145.
132. Kizil, C et al.,.(2012).Adult neurogenesis and brain re-generation in zebrafish. *Dev. Neurobiol.* 72: 429–461. doi:10.1002/dneu.20918.