Regulation of Caenorhabditis Elegans model in Alzheimer's Disease

Yuxuan Jing[1,*]

[1]Yew Chung International School – Secondary School, Hong Kong, 999077, China

Abstract. Alzheimer's disease (AD) is affecting numerous families and individuals around the world nowadays, as the exact reason is still undetermined. At this stage, developmental treatment displays a particularly significant role in relieving symptoms for the patients. Currently, the two most well-known factors that have impacts on the diagnosis of AD are the plaques and tangles formed from amyloid-beta and tau protein. Modelling for Alzheimer's disease is essential in understanding targeted aspects of the disease, while Caenorhabditis elegans (C.elegans) was chosen as a pivotal model. C.elegans presents dramatic priorities using orthologs for the study of AD, especially in examining the formation of the deposits and the regulations of specific gene expressions that result in this abnormality. This review discusses the properties, which C.elegans shows on the study of AD, and the achievements that have been approached using this model, as well as what other models are being tested by scientists. Properties of other models, which can overwhelm C.elegans, as well as the expectations for future modelling systems on AD are examined as well.

1 Introduction

Alzheimer's disease (AD) is one of the most prevalent chronic diseases of dementia caused by neurodegeneration, which usually leads to cognitive problems, such as memory decline and behaviour disorders. The first diagnosis of Alzheimer's disease traced back to 1901 when Alois Alzheimer (1864-1915) defined his patient Auguste Deter as the earliest Alzheimer's dementia. Approximately 44 million people around the world are suffering from various forms of dementia, specifically AD. Women and elderly (age>65) appear to undergo higher risks of showing symptoms of AD. In order to access further understanding and more advanced treatment on AD, scientific models have been applied in recent research. Caenorhabditis elegans (C.elegans) was introduced as a genetic model for developmental neurobiology a half-century ago. This transparent nematode worm has a relatively simple body structure with no respiratory or circulatory system, and can be found in the soil around the world easily. C.elegans has now been targeted for comprehensive approaches to AD, and some corresponding achievements are under processing. In this review, the expanded results of using C.elegans as a model to study AD will be discussed, as well as an illustration of the privileges and limitations of the model. The comparison between the up-to-date and old AD models will be elaborated with expectations on the implementation of new methods on AD treatments in the future.

2 Introduction of C.elegans

2.1 Body structure and lifespans

Caenorhabditis elegans (C.elegans) is a nematode with a significantly simpler body structure than mammals, especially humans. Its nervous system takes control of its whole body movement and information processing. However, neither specialized circulatory nor respiratory system [1] exists in C.elegans, as gas exchange is conducted by diffusion through cells directly without other metabolic pathways. Pharynx, as an essential organ in C.elegans, is responsible for multiple functions, and most importantly, feeding and deglutition [2] completed by the peristalsis of the pharynx. Two natural sexes occur in C.elegans [3], which are male (XO) with 1031 cells and hermaphrodite (XX) with 959 cells; they either self-fertilize or breed with males to reproduce; and up to 1000 offspring can be produced throughout the lifetime.

C.elegans has a short lifespan with a total adult life for up to three weeks. The normal life cycle has three main stages (Egg → Larvae → Adults) and four Larvae stages, which are designated as L1, L2, L3, and L4. C.elegans at Larvae stage is only 0.25 millimeters long, while adult C.elegans is still around 1 millimeter long [3], making it able to be analysed under the microscope. Under a favourable living environment, 55 hours is needed for C.elegans to grow from embryos to adults. When the living condition is unfavourable for the growth of C.elegans, such as shortage of food-supply or unsuitable temperature, C.elegans at the L2 stage might make the

* Corresponding author: yuxuan.jing18@sec.ycis-hk.com

decision of entering the Dauer stage [4] to delay the normal growth pace, in order to survive in harsh environments.

2.2 Nervous system of C.elegans

2.2.1 Specialized nervous system

The nervous system is located in the pharynx of C.elegans. Adults C.elegans have 302 neurons in total, with approximately ⅓ sensory neurons, ⅓ interneurons, and ⅓ motor neurons [5]. Each of the neurons is named by two or three capital letters; in some cases, combined with numbers if the neurons are from the same class. Neurons in C.elegans have a specific location in a lineage diagram, which is available to trace the history of each individual neuron [6]. Neurons in C.elegans are connected to each other by synaptic connections, and they can communicate with each other either through chemical synapses or electrical gap junctions.

Neuronal activities, including cellular and synaptic activities in C.elegans, are recorded by the genetically encoded fluorescent Calcium sensor called GcaMP [7]. This acts as an indicator, which creates an image of C.elegans neurons responding to the $Ca2^{+}$ signaling by showing its dynamic change.

2.2.2 Aging of nervous system in C.elegans

Aging brings irreversible transformations to the biological nervous system in organisms, even if in nematode worms, like C.elegans with only 302 neurons. Metabolism in C.elegans maintains the absorption and utilization of nutrients from its food, and the rate of metabolism declines as C.elegans get older. Although aging in C.elegans is an inducement for a chain of morphological changes, such as the distinct shrink of body size in the L4 stage, decreased organization of body wall muscle and pharynx, neurons are deemed to have the least microscopic change compared to other tissues in C.elegans. Main components of neurons still remain relatively complete and functional [8], however, detectable age-related changes of neurons still exist.

During C.elegans aging stage, degeneration of synaptic activities happens as the number of vesicles in synapses to receive signals from effector neurons decline [9]. This indicates that young worms have stronger functionality in sensing and responding to nerve stimulations. In addition, the number of branches in neurons gradually increases; one of the causes may be that neurons have lost the ability to control the neurites from growing excessively. More blisters are grown to make them appear to be beady and blebby as clarified in Figure 1[10], which normally happen in damaged or hypoxic neurons [11].

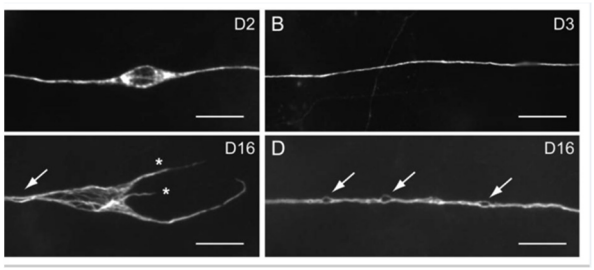

Fig. 1. Clear bubble-like lesions and abnormal branches of microtubules in the old neurites of C.elegans can be observed.

Neuronal aging in a human's brain is similar to a certain degree. Synaptic activities also show a significant decline with a decrease in the number of synapses; however, myelin phosphate layers coated around neurons start to shrink and dendrites become less brachy, which is on the contrary to C.elegans.

2.2.3 Calcium activity alternation in older neurons

Signals that trigger neuronal activities are controlled by the fluctuated intracellular calcium concentration. As age increases, calcium homeostasis is affected and becomes deregulated and could possibly damage neurons [12]. In older neurons, calcium concentration is higher than that in younger neurons due to the increase of calcium influx and calcium buffer [13]. In some neurons, higher contents of intracellular calcium medication lead to the overactivation of substances, such as glutamate receptors [14], causing the death of neurons. What is more, the imbalance of calcium homeostasis mechanisms also worsens the process of aging and neurodegeneration.

2.3 Behavioural activities existing in C.elegans

2.3.1 Common behaviours of C.elegans

Neurons differentiate to implement a variety of functions in the organism. Several behaviours in C.elegans can symbolise certain neuronal activities within different neuron types [15]. The most visible behaviour is movement and food feeding. C.elegans intuitively search for food, such as bacteria. Egg-laying is another typical behaviour of C.elegans, conducted by its reproductive system [16]. More complex behaviour includes fear and anxiety when C.elegans moves fast to avoid predators. Many other behaviours are affected when it's stimulated by the presence of predators; for instance, egg-laying is stopped or requires an extended period of time to complete [17].

2.3.2 Differ in behaviours in old and young C.elegans

At an advanced age, highly potentially that neurons may die due to abnormality of calcium concentration [18], leading to behavioural disorder. C.elegans presents dramatic declines of a wide range of its capabilities, including motor, perception, feeding, and reproduction.

The most significant change is the deterioration of motor ability as the worms get older. The speed of body-

moving declines progressively, together with a lower sense of direction and coordination [8]. If the body movement is quantitatively observed and measured as wave per minute, results have shown a gradual decrease in the frequency of waves as C.elegans get older. Along with the degradation of body movement is the decline of the sensory perception [19] to stimulations, such as favourable odors, food, and changing temperature. These can be explained by the loss of muscle structure and function, which leads to the deficits of the motor.

C.elegans displays weakened effectiveness in feeding capability at an advanced age. Its pharynx responsible for swallowing food becomes less motivated for rhythmic contraction [20], and finally ceases at around Day 12 during its lifetime.

The ability for reproduction in hermaphrodites gradually loses during aging [19]. Tissues of the reproductive system in old worms experience degeneration which leads to restraint in the production of sperms, allowing worms in adulthood to produce less offspring until no progeny can be produced any more to a certain point. Several similar aging behaviours are presented in mammals. Behavioural study in C.elegans helps to explore the proceeding mammalian aging research since certain drugs act on regulating aging in C.elegans might be developable in human age-related diseases (Alzheimer's disease, Parkinson's disease, etc.) as well [21].

3 Introduction of Alzheimer's Disease

3.1 History of Alzheimer's disease Research

Dementia had long existed in history even before people had recognized this as a disease related to increasing age. However, Alzheimer's disease, as the typical symptom of dementia, which is the leading threat of health among the elderly, was not named after Alois Alzheimer (1864-1915) announced the first case as "presenile dementia" towards a patient called Auguste Deter in the early twentieth century [22]. Auguste showed cognitive disorders, such as memory decline, delusion, and psychological social disability, so she entered a mental hospital for treatment. The development of this disease started to step out by studying Auguste's brain tissue after she passed away. Emil Kraepelin (1856-1926), Alzheimer's teacher and coworker, had officially named this type of dementia "Alzheimer's Disease" in 1910 [23]. After this, the terminology of "Alzheimer's disease" started to be authorized in the field of medicine progressively, and in the late 1970s, the awareness of AD spread out as it had turned into one of the major concerns of old people.

In 1984, a critical protein—amyloid-β protein was confirmed to exist in the blood of patients with Alzheimer's disease [24]. However, patients with Down Syndrome also perform the same characteristic of having Aβ protein [25]. On a large probability that these two types of patients grow dementia with plaques and tangles during midlife, where chromosome 21 reached a position of prominence back then. Shortly after the discovery of amyloid -β protein, the further research of "tangles" brought out another tau protein [26] that was defined as linked to the progression of AD. Abnormally buildup of tau protein instead of binding to microtubules in patients' brains caused the formation of tangles of neurofibrillary, damaging the brain cells and blocking them from communicating with each other. Research treatment methods for AD hence started to draw scientists' attention, and in the 1990s, several significant discoveries were published, including the gene locus for dominant, and minor Alzheimer's disease which are presenilin 1 [25] (PSEN1) and presenilin 2 (PSEN2) on chromosome 14 and 1 respectively; apolipoprotein APOE4 allele had also been demonstrated as a factor that causes the onset of Alzheimer's disease. It is still a challenge when it comes to the effective cure for Alzheimer's disease, an increasing number of people have been reported with AD, especially in some modern countries where there is a larger dependency ratio and a higher proportion of aged people [27].

3.2 Molecular mechanism of AD

The clear biological cause for Alzheimer's disease is still under research, while the buildup of excess protein in the brain can be the main trigger. However, from previous research, some factors have been speculated and said to be risky for AD.

Aging is construed as an essential cause that might lead to this disease. Statistics have shown that the number of people with AD grows as the age increases; almost one-third of the population with an age of 85 have the potential to suffer from AD [27]. This is related to aging in the brain and nervous systems, such as shrinking of the size of the brain, structural change towards neurons and brain size, and fluctuations in neuronal activities.

Genetics is considered to be another important causation of AD. Currently, studies have shown that three gene mutations are associated with the early-onset Alzheimer's disease, which occurs between middle-aged people, usually below 60 years old. The amyloid-β protein on chromosome 21, PSEN1 on chromosome 14, and PSEN2 on chromosome 1 [28] are determined to be spotlighted for AD to happen, as they may result in abnormal formation of brain cells. These gene mutations have the probability of being passed on to the offsprings [29], followed up by the onset chain of AD in family history. However, for the late-onset Alzheimer's disease that is being the most common type happening among people aged over 65 years old, the APOE4 allele is what makes an impact [30]. APOE4 has three main forms, APOE ε2, APOE ε3, and APOE ε4. APOE ε4 is the allele that may lead to an increased risk of having AD, but this does not make the inevitable development of the disease. Each person keeps two of the three alleles; both of them are inherited from paternal and maternal genes respectively.

Other environmental factors are either acquired from daily life or inherent, such as smoking, obesity, high blood pressure, and diabetes, which are being typically risky towards the disease [31]. Down Syndrome is also partly related to the risk of Alzheimer's disease because of the

abnormal trisomy chromosome 21 [25]. Apart from the ineluctable factors of aging and inheritance, maintaining a healthy lifestyle will bring no harm but benefits.

3.3 Common symptoms and effect of AD

Alzheimer's disease is a neurodegenerative disease that the neurons and brain cells eventually get damaged and die. As this change is irreversible, AD brings permanent harm to the brain and nervous system. The hippocampus will be attacked at first, followed by the lateral ventricles and amygdala, causing dysfunction of the brain cells [32]. AD also influences the synaptic activity between neurons, so that neurons lose the ability to communicate. As a result, shrinkage of the brain becomes the corollary due to the sequential damage to the brain.

AD does not emerge in one night, while the progressive loss of a variety of abilities and effects on daily life can be seen in the long term. In a short word, symptoms of AD are categorized as three main stages with seven smaller stages. The early stage is when the patient does not show overfull symptoms or has mild symptoms [33]. Memory loss accompanied with shorter memory circle towards new names, less accuracy towards choices of words, and less capability towards being organized or planned are the summarized behavior of patients at this stage. However, assisted living is not yet needed if the patient is keeping a healthy and ordered living mode. Correspondingly, stage one to stage four are considered as mild Alzheimer's disease, but the degree of being disorganized and forgetful gradually increases.

Up to the moderate stage of AD, which is also the longest stage before further deterioration of illness, relatively severe symptoms start to flourish. Personal information cannot be recalled, such as a living address, phone number, or even the name of close relatives. Self-care capability shows an obvious drop, and patients start to suffer from gatism, sleeping with day and night inverted, difficulty in finding the way home, and so on. However, preserved skills, most common as reminiscing, might leave inside of the patient's brain for a longer period of time even though the symptoms seem to be getting worse [34]. Stage five and six are categorized to be moderate to severe stages of AD. However, 6c, 6d, and 6e stages exist in stage 6, indicating the process of the ability to toileting independently gradually decreases, eventually resulting in fecal incontinence.

Stage 7, also known as severe Alzheimer's disease, is where patients cannot access daily assistance without the help of caregivers [33]. Degradations occur in motor, cognitive, and memorizing abilities, and patients can neither manage to finish a completed sentence nor walk or sit. Several neurological changes affect the patient by bringing back some primitive reflexes [35] that should only apply to infants. Some noticeable reflexes are suck reflex and grasp reflex, but with higher strengths and stronger affections. Pneumonia, as well as other common infections, maybe the usual finales for an AD patient [36]. They are too weak in stage 7 so that patients are likely to be affected by many other diseases.

3.4 Treatments of AD

Treatments for Alzheimer's disease is always a massive concern for scientists. Currently, no cure for AD has been identifiably feasible, although some major associated factors leading to AD might have already been drawing the attention. At present, drugs have been taken into use only to relieve symptoms, slow down memory loss, and logical thinking skills. However, factors, such as stages of the disease, temporary efficacy, targeting on a specific protein are still restricting the effectiveness of these drugs; no tangible methodology can get rid of the degeneration and death of brain cells.

Existing treatments target the plaques caused by protein beta-amyloid and the tangled formed by tau protein [37]. Solanezumab is a monoclonal antibody that acts on beta-amyloid proteins to prevent the formation of plaques. However, solanezumab does not show much efficiency when it comes to moderate or severe AD patients [38]. Therefore, protein Fyn was taken into consideration as it has the property to react with beta-amyloid protein [39]. The abuse is that Fyn is stimulated to become over-activated and can potentially destroy synaptic activities in the brain. Without taking beta-amyloid protein off from the list, scientists started to explore other methods with tau protein simultaneously. Preventing tau from tangling with specific inhibitors is the key [40]. Similarly, inhibitors or vaccines are still in the testing phase. On May 28th, 2020, tauvid (flortaucipir F18), a radioactive diagnostic agent, was officially approved by the U.S. Food and Drug Administration for intravenous injection [41]. Tauvid images the tau tangles in the brain by estimating the distribution of tau neurofibrillary tangles using positron emission tomography (PET) imaging. It's the first authorized reagent after the approval of amyloid imaging for beta-amyloid protein pathology. These reagents are biomarkers towards the development of AD. They provide support for the diagnosis of the condition of the patient; meanwhile, the substantive achievement on the research of Alzheimer's disease.

4 C.elegans as a Scientific Model for Alzheimer's Disease

4.1 Reasons why need modelling for Alzheimer's disease

Scientific models always play a vital role in understanding further about human disease. Modelling is what embodies a core scientific phenomena or abstract expression using specific targets that are able to show certain properties.

Alzheimer's disease has been through decades of research on its therapeutic methods, but it still seems far to approach. Scientists are expecting models, specifically referring to animal models, can take a step further in addressing properties of AD, and therefore, testing possible therapies.

On the basis of the abnormal shape and size of the brain of AD patients, in particular, the shrivel of the hippocampus (part of the brain which in charge with the

memory), and accumulation of amyloid-β protein and tau protein, animals that possess so-called similar brains as humans or a relatively sound nervous system become hallmarks when determining biological models of AD. On top of that, life span, behavioural expression, and social contexts of that species also take into account. For instance, laboratory mice are always an essential starting point when conducting new research progresses. However, a full overview of AD is harder to explore in a single mice model, and it also pulls out the question of whether mice could present with targeted AD symptoms [42]. In the meantime, it takes several years to observe the whole aging process of a mouse.

Overall, although some factors have been affirmed to affect the disease, it's not possible to examine the neuronal or cerebral impacts in humans limited by unknown risks and humanitarianism. Therefore, animal models are widely introduced for scientists to look into.

4.2 Advantages of choosing C.elegans as a model for Alzheimer's disease

4.2.1 What attributes make C.elegans representative as an effective scientific model

A half-century ago, Caenorhabditis elegans (C.elegans) was introduced as a genetic model for developmental neurobiology. In 1977, C.elegans was defined as a useful system for feasible aging studies [43], and since then, it has become significant for exploring related neurobiology problems in higher complex organisms like humans. This transparent nematode worm has a relatively simple body structure with no respiratory or circulatory system, and can be found in the soil around the world easily, which makes C.elegans more manageable in laboratory works.

C.elegans can also be an economical model for scientific research in laboratory works. It does not require a specific environment to survive, whereas bacteria are the main source to feed it. Humans have 86 billion neurons, which is relatively difficult for studying the aging of neurons in humans. Therefore, this 1mm-long transparent microscopic nematode with only 302 neurons and around 7000 synapses, has been characterized as a powerful scientific model organism for the development of aging studying for the benefits of its short lifespan, wide distribution in soil worldwide, and easy identification of individual neurons.

4.2.2 Priorities when C.elegans is used to study Alzheimer's disease and linkage between this model and Alzheimer's disease

4.2.2.1 Calcium hypothesis related to brain aging and the significance of APP gene expression

C.elegans has been used as a model for several neuronal diseases in humans caused by calcium dysregulation. "Calcium hypothesis of Alzheimer's disease" was proposed to state that disorder and dysfunction of neurons are triggered when normal intracellular calcium signaling is beyond control, and calcium homeostasis is altered, which results in cognitive and memory deficit of the brain [44]. Imbalance of calcium homeostasis can be associated with Aβ and tau accumulation, as well as certain gene mutations that act on Calcium signaling. Consequently, these gene mutations lead to neurodegenerative diseases, typically AD.

The amyloid precursor protein (APP) is an integral transmembrane protein that can be cleaved by certain secretases [45]. APP has been defined as one of the major proteins that are correlative to AD. As a result, a large extracellular fragment is formed by the cleavage of β-secretase, while the γ-secretase releases a small intercellular fragment [46]. The Aβ peptide is the product from the cleavage of APP using these two screatases. The mutations of APP or presenilin proteins (PS1 or PS2) from PSEN1 and PSEN2 genes are the main contributors to Alzheimer's disease, while PS1 and PS2 are counted as portions of γ-secretase [45].

4.2.2.2 Apl-1 function in C.elegans

C.elegans are discovered to be orthologous with the APP gene with mammals. The examination of apl-1, the only APP-related gene in C.elegans [47], provides multipurpose engagement in perceiving the functionality of APP, whereas the deletion of APP directly has a direct relation to the accumulation of Aβ peptide.

The sequences of Apl-1 genes are highly alike compared to that of humans [48]: E1 and E2 domains share approximately 50% similar sequences with humans, and the transmembrane domain emerges 71% similarity. However, the Apl-1 gene in C.elegans does not produce Aβ sequence [47], allowing this model capable of being tested about the existence and deposition of Aβ sequence on muscle walls when the worm gets paralyzed.

Previous research has confirmed that knockout of APP morphological defects in mice, while multiple knockouts of APP results in an absolute high lethality [49]. Similarly, Apl-1 shows equivalent outcomes when it's inactivated in C.elegans. During the Larvae stage, C.elegans is observed to be mortal revealed from the inactivation of Apl-1, highlighting the indispensability of APP family in the upgrowth journey of C.elegans, especially molting cycle and motor ability in early stages [48]. Figure 2 [48] shows some morphological defects in C.elegans caused by the abnormality of Apl-1 expression.

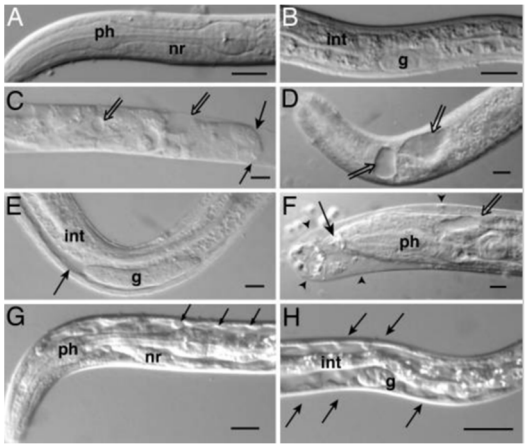

Fig.2. Ths most significant change in the appearance of the animal is the vacuoles around the organs and the translucent skin, specifically shown in section G and H. There is also incomplete or malformed growth of organs during early stages of C.elegans, as shown in section C and section D.

In addition to this, sel-12, as a presenilin gene of C.elegans, has the property of regulating cleavage of apl-1 [44]. Sel-12 homology and human homology resemble in sequencing, and sel-12 mutation alters the expression of apl-1. However, it generates degeneration of the neurons and deregulation of Calcium homeostasis.

4.3 Achievements or progress on developing Alzheimer's disease using this model

4.3.1 Crucial developmental achievements on this model

Establishment of Apl-1 gene expression in C.elegans has a relationship not only with growing at the larval stage, but the developmental process of AD. Specific encoded genes were determined to be involved in the development of AD and aggregation of Aβ peptide, including ttbk-2, daf-16, and unc-49 [50]. These genes have orthologs with humans that further research can be conducted to allow possible therapeutic methods on AD.

Investigations on specific Aβ gene expressions, Aβ1-42 and Aβ3-42 are also the focus of researchers [45]. Several genes orthologs to human heat shock (HSP) have direct interaction with Aβ3-42 expression in C.elegans. For Aβ1-42, a particular example is Hsp90, where autonomously protects neurons from toxic effects of Aβ(1-42) expression. Using the C.elegans model, researches defined that Hsp90 achieves this by regulating, especially conveying inhibitions to its own chemical activities [51]. What's more, Aβ aggregation situation can be controlled when Hsp90 expression is activated through transcellular chaperone signalling (TCS) by the cell, even if it's a distal cell [52]. However, TCS is contingent on the neurotransmitting process of glutamatergic, when the functionality of glutamate is also regulated by Hsp90. What interests scientists is whether Hsp90 might be restrictive and critical towards the treatment of AD, of being a regulator, and takes charge of glutamatergic neuronal signalling.

4.3.2 Recent C.elegans-associated study on AD

It has been confirmed that metal homeostasis significantly affects the deposition of Aβ, especially Copper and Zinc. [53]. Metal modification is also one of the ideal approaches to control AD pathology. Recently, metallothionein (MT), which proteins of this family are functioning for regulating metal homeostasis [54], is observed to increase in C.elegans with Aβ protein. As aging C.elegans experience a fluctuation in metal level, aggregation of Aβ is speeded up. MT takes part in transporting excess metal and reducing cellular toxicity through metal-thiolate bonds [55], ameliorated the formation of Aβ, and the neurodegenerative effects that are brought out. This has further tested the feasibility of drugs aiming to regulate metal levels, marking a bright direction in advanced research.

Euphorbia pulcherrima, the species from diverse spurge family, its extract eupulcherol A (1) has been lately tested on C.elegans model [56]. One severe AD symptom in C.elegans is paralysis, while this compound shows remarkable prolongation in delaying the paralysis in C.elegans model, making this a potential indicator of delayed onset of Alzheimer's disease.

5 Limitations and Expectations of C.elegans for Studying Alzheimer's Disease

5.1 Comparison of developmental models that have been chosen or tested by scientists

Research in treatments for AD has been carried out for decades. However, barely drugs have been officially available in the market. Animal models are built to understand more about the disease, while mice that undergo genetic modification named PD-APP mice [57] promoted notable achievements. The phenomenon of increasing plaques and tangles, cognitive problems, and faster rate of the deposition of Aβ are as much similar to the symptoms of AD in humans [58]. In the late 1990s, an experimental vaccine was tested on PD-APP mice, aiming to use monoclonal antibodies for the clearance of Aβ [59]. However, there was no obvious improvement in the patient's memory after the disappearance of Aβ.

Scientists hence started to look into invertebrate models that minimize the complexity of animals to concentrate the focus of researching. Drosophila and C. elegans [60] are both widely used models when studying neurodegenerative diseases. Compared to PD-APP mice, these two models give out faster responses due to a shorter life span, simpler body structure, and relatively fixed behavioural activities. Numerous orthologs genes with humans exist in C.elegans, and Drosophila that the specific property and regulation of that expression related to AD can be achieved. Scientists might be able to test different mutations by creating transgenic mice, but the experiments would take a longer time, and more complex influencing factors would be taken into consideration. There are risks that the symptoms the mice possess do not match the biological disease might be misleading.

The vertebrate model, Zebrafish, is gradually occupying an important position in modelling for AD nowadays. It shows significant advances in comparison to rodent models [61]. During biological processes, Zebrafish shows clear duality from larva to adult. When analyzing the properties of Zebrafish, the drawbacks are said to be filled where invertebrate models are not able to perform complicated responses due to the lack of complexity of the nervous system. Orthologs of APP have been identified in Zebrafish; this brings out strengthened capabilities for the study of Aβ and tau proteins in the present case.

5.2 Limits of using C.elegans compared to old models or methods

The nematode C.elegans is one of the invertebrate models and has been specifically exploited for AD. However, there are limitations, according to this scientific model. It's short lifespan definitely carries out the advantage of quick response towards a target, but it also restricts the concept when designing core researching questions, such as C.elegans might not be able to show the long-term effect of clearance of Aβ aggregation. In addition, only 302 existing neurons are not comparable to 86 billion neurons in the human brain. Although experiments on orthologs can be conducted to investigate the possessional attributes, this result can be too microcosmic as there might be alterations when putting into a macro condition like the human nervous system. Given the discussion of complexity, C.elegans can lay the foundation for the treatment research of AD, while following up experiments on other more complex biological models require more to achieve. For instance, Zebrafish is able to present more advanced behaviours, social ability, and even memorization compared to C.elegans, making it a superior indicator towards aging research.

5.3 The progress of research based on the study of C.elegans on Alzheimer's disease

C.elegans model is expected to be improved in the near future to particular propositions and aspects. For example, as the definition of Aβ becomes a label for the causes of AD, a specific neuronal circuit that loses resistance on Aβ is on the waiting list for further evaluation. At the same time, scientists are curious about the glutamatergic neuronal function maintenance under the protection of Hsp90. Therefore, a more advanced C.elegans model is likely to come out with more comprehensive monitoring on the process of aging or neuronal signaling.

Apart from the current findings that have been defined on the basis of modelling C.elegans for AD, other efficient models from different species are at the trial stages. Macaques are good choices to study aging and AD, as marmoset animals do show tangling and Aβ deposits as aging increases. However, the uncertainty of this model is still underestimated since marmosets were not prevalently introduced in the scientific literature. A marmoset model [62] of Alzheimer's disease has been created with several inserted mutations. People are starting to test on species that show a high proportion of similarities in immune systems since scientists would like to approach as close as the mysteries behind AD. However, using this type of model is always controversial, ethnically and economically.

In the future study, vaccines or injections might be potentially usable as there is some fresh news discussing the newly developed injections. Drugs still maintain the main repressor for mild to severe AD patients today, and Bryostatin-1 has just been approved to conduct with further exploration in the absence of Namenda® (memantine) on May 28th. In the meantime, nilvadipine that helps to relieve the effects of memory loss and cognitive issues is in the Phase 3 clinical trial now. The prospect of finding the real cause of Alzheimer's disease still seems to be a costing and challenging project worldwide right now, while the development of the treatments to reduce the pain of patients who are currently suffering from AD is presenting the world with hopeful forecasts.

6 Conclusion

Alzheimer's disease is one of the major aging diseases that are indisputably affecting 44 million people in the world right now. The root cause of AD still remains unknown, but existing treatments can be efficient in slowing down the deterioration of the condition, targeting different stages of the patients. When it comes to the long term effect, correlating side effects of pharmaceutical drugs and inevitable degeneration of the brain are still issues remaining with the disease.

AD is examined to be associated with two major proteins, amyloid-beta and tau. Plaques and tangles are products of the abnormal formation of these two proteins. In terms of developmental research of AD, modelling is a vast component and has demonstrated eminent progress. Caenorhabditis elegans is a significant scientific model for studying AD. The most significant advantages of this model owe to access to a clear overview and understanding of its C.elegans' simple body structure and nervous system. Investigating gene expressions by targeting orthologs in C.elegans is one of the major favourites in studying AD using this model. The only APP-related gene with highly sequencing mutuality as APP protein in humans is named after apl-1. By testing on apl-1 gene in C.elegans, APP has been determined as a dominant factor resulting in the aggregation of Aβ deposits.

Several gene mutations in C.elegans are defined to be able to regulate the expression of apl-1, such as the presenilin gene ---- sel-12, allowing it to become possible to adjust the expression of APP in AD patients. Aβ gene expressions, including Aβ1-42 and Aβ3-42 in this model also brought out highlighted achievements on exploration on AD. For example, the regulation of Hsp90 through releasing neuronal signals has shown efficiency in controlling the expression of Aβ1-42. On top of that, the metallothionein (MT) which can bring remission to the toxicity of Aβ protein, as well as the extract eupulcherol A from Euphorbia pulcherrima functioning in delaying

the paralysis of C.elegans infected by AD, are included in the very latest research.

However, compared to other animal models, such as PD-APP mice and Zebrafish, C.elegans shows distinct advantages and drawbacks. Due to the lack of complexity of its brain, C.elegans makes research easy to carry out in laboratory works. It's restrictive at the same time, especially when the ultimate target is to study the human brain, making the conclusions drawn from the C.elegans model fluctuant. By contrast, a vertebrate model with a more complex brain and similar orthologs with humans can be more suitable.

Recently, some new technologies have been put into practice. Nowadays, the density of tau tangle formation in the patient's brain can already be successfully pictured. Apart from this, trials of nilvadipine and research of Bryostatin-1 are also under progress, creating a bright future for the development of AD, as well as the development of human-like scientific models.

References

1. Bretscher, A. J., Kodama-Namba, E., Busch, K. E., Murphy, R. J., Soltesz, Z., Laurent, P., & de Bono, M. (2011).
2. Song, B. M., & Avery, L. (2013, January). The pharynx of the nematode C. elegans: a model system for the study of motor control. In Worm (Vol. 2, No. 1, pp. 1920-31). Taylor & Francis.
3. Ann K. Corsi, B. W., and Martin Chalfie (2015). "A Transparent window into biology: A primer on Caenorhabditis elegans."
4. Riddle DL, B. T., Meyer BJ, et al. (1997). *C. elegans II.*
5. Hobert, O. (2005). "Specification of the nervous system."
6. J.E. Sulston, E. S., J.G. White and J.N. Thomson (1983). "The Embryonic Cell Lineage of the Nematode Caenorhabditis elegans."]
7. Kerr, R. A. (2005). "Imaging the activity of neurons and muscles."
8. James J. Collins, C. H., Stacie Hughes · Kerry Kornfeld (2007). "The measurement and analysis of age-related changes in Caenorhabditis elegans."
9. Marton Lorant Toth, I. M., Leena Shah, Aatish Bhatia, Kevin Lu, Amish Talwar, Haaris Naji, Carolina Ibanez-Ventoso, Piya Ghose, Angela Jevince, Jian Xue, Laura A. Herndon, Gyan Bhanot, Chris Rongo, David H. Hall, and Monica Driscoll (2012). "Neurite Sprouting and Synapse Deterioration in the Aging Caenorhabditis elegans Nervous System."
10. Chiu-Ying Peng, C.-H. C., Jiun-Min Hsu, and Chun-Liang Pan (2011). "C. elegans model of neuronal aging."
11. Heehwa G. Son, O. A., Eun Ji E. Kim, Sujeong Kwon, and Seung-Jae V. Lee (2019). "Age-dependent changes and biomarkers of aging in Caenorhabditis elegans." from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6413654/.
12. Tavernarakis, V. N. a. N. (2012). "Calcium homeostasis in aging neurons."
13. M. Matthew Oh, F. A. O., Jack Waters and John F. Disterhoft (2013). "Altered Calcium Metabolism in Aging CA1 Hippocampal Pyramidal Neurons."
14. Javier Alvarez, P. A.-I., Paloma García-Casas, Rosalba I. Fonteriz, and Mayte Montero (2020). "The Role of Ca2+ Signaling in Aging and Neurodegeneration: Insights from Caenorhabditis elegans Models."
15. Chao., A. C. H. a. M. Y. (2010). Chapter 1From Odors to Behaviors in Caenorhabditis elegans. *The Neurobiology of Olfaction*. M. A.
16. Koelle, M. (2005). Assessing defective or constitutive egg-laying. *Wormbook*. A. C. Hart.
17. Zheng Liu, M. J. K., Christopher D. Chute, Amy K. Pribadi, Sarah G. Leinwand, Ada Tong, Kevin P. Curran, Neelanjan Bose, Frank C. Schroeder, Jagan Srinivasan & Sreekanth H. Chalasani (2018). "Predator-secreted sulfolipids induce defensive responses in C. elegans."
18. Laura Berliocchi, D. B., and Pierluigi Nicotera (2005). "Ca2+ signals and death programmes in neurons."
19. Heehwa G. Son, O. A., Eun Ji E. Kim, Sujeong Kwon, and Seung-Jae V. Lee (2019). "Age-dependent changes and biomarkers of aging in Caenorhabditis elegans."
20. David K Chow, C. F. G., Josiah L Johnston, Ilya G Goldberg, Catherine A Wolkow (2006). "Sarcopenia in the Caenorhabditis elegans pharynx correlates with muscle contraction rate over lifespan."
21. V.Lee, E. J. E. K.-J. (2019). "Recent progresses on anti-aging compounds and their targets in Caenorhabditis elegans."
22. Hyun Duk Yang, D. H. K., Sang Bong Lee, Linn Derg Young (2016). "History of Alzheimer's Disease."
23. Pei-Pei Liu, Y. X., Xiao-Yan Meng & Jian-Sheng Kang (2019). "History and progress of hypotheses and clinical trials for Alzheimer's disease."
24. George G. Glenner, C. W. W. (1984). "Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein.".
25. Natalie S. Ryan, M. N. R., Nick C. Fox (2015). "Alzheimer's disease in the 100 years since Alzheimer's death."
26. Khalid Iqbal, F. L., Cheng-Xin Gong, and Inge Grundke-Iqbal (2011). "Tau in Alzheimer Disease and Related Tauopathies."
27. Corrada, C. H. K. a. M. M. (2012). "Alzheimer's and Dementia in the Oldest-Old: A Century of Challenges."

28. Hélène-Marie Lanoiselée, G. N., 3 David Wallon, Anne Rovelet-Lecrux, Morgane Lacour, Stéphane Rousseau, Anne-Claire Richard, Florence Pasquier, Adeline Rollin-Sillaire, Olivier Martinaud, Muriel Quillard-Muraine, Vincent de la Sayette, Claire Boutoleau-Bretonniere, Frédérique Etcharry-Bouyx, Valérie Chauviré, Marie Sarazin, Isabelle le Ber, Stéphane Epelbaum, Thérèse Jonveaux, Olivier Rouaud, Mathieu Ceccaldi, Olivier Félician, Olivier Godefroy, Maite Formaglio, Bernard Croisile, Sophie Auriacombe, Ludivine Chamard, Jean-Louis Vincent, Mathilde Sauvée, Cecilia Marelli-Tosi, Audrey Gabelle, Canan Ozsancak, Jérémie Pariente, Claire Paquet, Didier Hannequin, Dominique Campion, and collaborators of the CNR-MAJ project (2017). "APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases."
29. Genetic Alliance, The New York-Mid-Atlantic Consortium for Genetic and Newborn Screening Services(2009). *Understanding Genetics: A New York, Mid-Atlantic Guide for Patients and Health Professionals.*
30. Chia-Chen Liu, T. K., Huaxi Xu, and Guojun Bu (2013). "Apolipoprotein E and Alzheimer disease: risk, mechanisms, and therapy."
31. Marcos Vinícius Ferreira Silva, C. d. M. G. L., Luan Carlos Vieira Alves, Leonardo Cruz de Souza, Karina Braga Gomes Borges & Maria das Graças Carvalho (2019). "Alzheimer's disease: risk factors and potentially protective measures."
32. Pierrick Coupé, J. V. M., Enrique Lanuza, and Gwenaelle Catheline (2019). "Lifespan Changes of the Human Brain In Alzheimer's Disease."
33. H Förstl, A. K. (1999). "Clinical features of Alzheimer's disease."
34. W W Beatty, P. W., R L Adams, E W Allen, D A Wilson, J R Prince, K A Olson, K Dean, D Littleford (1994). "Preserved cognitive skills in dementia of the Alzheimer type."
35. Alfredo Damasceno, A. M. D., Daniel F C Mazo, João F D Zullo, Patricia Scherer, Ronny T Y Ng, Benito P Damasceno (2005). Primitive reflexes and cognitive function. *Arq Neuropsiquiatr*.
36. Manabe, T., Fujikura, Y., Mizukami, K., Akatsu, H., & Kudo, K. (2019). Pneumonia-associated death in patients with dementia: A systematic review and meta-analysis.
37. Jason Weller, a. A. B. (2018). "Current understanding of Alzheimer's disease diagnosis and treatment."
38. Lawrence S. Honig, B. V., Michael Woodward, Mercè Boada, Roger Bullock, Michael Borrie, Klaus Hager, Niels Andreasen, Elio Scarpini, Hong Liu-Seifert, Michael Case, Robert A. Dean, (2018). "Trial of Solanezumab for Mild Dementia Due to Alzheimer's Disease."
39. Nygaard, H. B. (2017). "Targeting Fyn Kinase in Alzheimer's Disease."
40. Jürgen Götz, A. I., and Lars M Ittner (2012). "Tau-targeted treatment strategies in Alzheimer's disease."
41. Hoffman, M. (2020). "First Tau Imaging Agent for Alzheimer Disease FDA-Approved."
42. Masashi Kitazawa, R. M., and Frank M. LaFerla (2015). "Transgenic Mouse Models of Alzheimer Disease: Developing a Better Model as a Tool for Therapeutic Interventions."
43. Berridge, M. J. (2009). "Calcium hypothesis of Alzheimer's disease."
44. Javier Alvarez, P. A.-I., Paloma García-Casas, Rosalba I. Fonteriz and and M. Montero (2020). "The Role of Ca2+ Signaling in Aging and Neurodegeneration: Insights from Caenorhabditis elegans Models."
45. Adanna G. Alexander, V. M. a. C. L. (2014). "Use of Caenorhabditis elegans as a model to study Alzheimer's disease and other neurodegenerative diseases."
46. Can Zhang, A. B., Jason R. DiVito, Jesse A. Stevenson, Donna Romano, Yuanlin Dong, Zhongcong Xie, and Rudolph E. Tanzi (2012). "Amyloid-β Production Via Cleavage of Amyloid-β Protein Precursor is Modulated by Cell Density."
47. I Daigle, C. L. (1993). "apl-1, a Caenorhabditis elegans gene encoding a protein related to the human beta-amyloid protein precursor."
48. Angela Hornsten, J. L., Shruti Fadia, Richard Malins, Lawrence Ha, Xiaomeng Xu, Isabelle Daigle, Mindy Markowitz, Gregory O'Connor, Ronald Plasterk, and Chris Li (2007). "APL-1, a Caenorhabditis elegans protein related to the human β-amyloid precursor protein, is essential for viability."
49. Maya A. Koike, A. J. L., Jonathan Pham, Elaine Nguyen, James J. Yeh, Rombod Rahimian, Bruce J. Tromberg, Bernard Choi, Kim N. Green, Frank M. LaFerla (2012). "APP Knockout Mice Experience Acute Mortality as the Result of Ischemia."
50. Rasoul Godini, R. P., Hossein Fallahi (2019). "Caenorhabditis Elegans Hub Genes That Respond to Amyloid Beta Are Homologs of Genes Involved in Human Alzheimer's Disease."
51. Jiang-Rong Ou, M.-S. T., An-Mu Xie, 3 Jin-Tai Yu, and Lan Tan (2014). "Heat Shock Protein 90 in Alzheimer's Disease."
52. Dr Patricija van Oosten-Hawle, P. N. C. a. D. S. C. (2020). "An improved C. elegans model of Alzheimer's disease to monitor neuronal signalling activity."
53. Tanzi, A. I. B. a. R. E. (2008). "Therapeutics for Alzheimer's disease based on the metal hypothesis."
54. Yonggang Wang, X. M., Jian Sun, LuCai (2016). Chapter 6 - Oxidative Stress in Diabetes: Molecular Basis for Diet Supplementation. *Molecular Nutrition and Diabetes.*
55. Dagmar Pretsch, J. M. R., Axel Schmid, Miroslav Genov, Teresa Wöhrer, Liselotte Krenn, Mark Moloney, Ameya Kasture, Thomas Hummel &

Alexander Pretsch (2020). "Prolongation of metallothionein induction combats Aß and α-synuclein toxicity in aged transgenic Caenorhabditis elegans."

56. Chun-Xue Yu, R.-Y. W. a., Feng-Ming Qi, Pan-Jie Su, Yi-Fan Yu, Bing Li, Ye Zhao, De-Juan Zhi, Zhan-Xin Zhang and Dong-Qing Fei (2020). "Eupulcherol A, a triterpenoid with a new carbon skeleton from Euphorbia pulcherrima, and its anti-Alzheimer's disease bioactivity."
57. James A. Richardson, D. K. B. (2002). "Mouse Models of Alzheimer's Disease: A Quest for Plaques."
58. Green, F. M. L. a. K. N. (2012). "Animal Models of Alzheimer Disease."
59. Masliah, C. A. L. a. E. (2010). "Can Alzheimer disease be prevented by amyloid-β immunotherapy?".
60. Claudia Saraceno, S. M., Elena Marcello, Silvia Pelucchi and Monica Di Luca (2013). "Modeling Alzheimer's disease: from past to future."
61. Morgan Newman, E. E., and Michael Lardelli (2014). "Using the zebrafish model for Alzheimer's disease research."
62. Ingrid H Philippens, P. R. O., Guus Baarends , Maja Johansson , Ed J Remarque , Magnus Doverskog (2017). "Acceleration of Amyloidosis by Inflammation in the Amyloid-Beta Marmoset Monkey Model of Alzheimer's Disease.