Genetic Dissection of Alzheimer’s Disease Using Drosophila Models

Youngjae Jeon 1, Jae Ha Lee 1, Byoungyun Choi 1, So-Yoon Won 2,* and Kyoung Sang Cho 1,*

1 Department of Biological Sciences, Konkuk University, Seoul 05029, Korea; hhghhh@naver.com (Y.J.); aquari2@naver.com (J.H.L.); quddbs316@konkuk.ac.kr (B.C.)

2 Department of Biochemistry, Chungbuk National University College of Medicine, Cheongju 28644, Korea

* Correspondence: sywon@chungbuk.ac.kr (S.-Y.W.); kscho@konkuk.ac.kr (K.S.C.); Tel.: +82-10-3688-5474 (S.-Y.W.); +82-2-450-3424 (K.S.C.)

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Abstract: Alzheimer’s disease (AD), a main cause of dementia, is the most common neurodegenerative disease that is related to abnormal accumulation of the amyloid β (Aβ) protein. Despite decades of intensive research, the mechanisms underlying AD remain elusive, and the only available treatment remains symptomatic. Molecular understanding of the pathogenesis and progression of AD is necessary to develop disease-modifying treatment. Drosophila, as the most advanced genetic model, has been used to explore the molecular mechanisms of AD in the last few decades. Here, we introduce Drosophila AD models based on human Aβ and summarize the results of their genetic dissection. We also discuss the utility of functional genomics using the Drosophila system in the search for AD-associated molecular mechanisms in the post-genomic era.

Keywords: AD model; Alzheimer’s disease; amyloid β; Drosophila; functional genomics

1. Introduction

1.1. Genetics of Alzheimer’s Disease

Alzheimer’s disease (AD) is a progressive neurological disorder that results in irreversible loss of neurons, particularly in the cortex and hippocampus. As of 2019, over 50 million people worldwide have AD or a related dementia [1]; which leads to death within three to nine years after diagnosis [2].

In the brain of an AD patient, amyloid beta (Aβ)-containing senile plaques and neurofibrillary tangles (NFTs), the aggregates of hyperphosphorylated tau protein, are observed, which are the main hallmarks of AD [3]. Therefore, Aβ, the cleaved form of amyloid precursor protein (APP), and tau, a microtubule-binding protein, have been suggested to be important causative molecules in the pathology of AD [4,5]. Aβ can aggregate to form flexible soluble oligomers that are toxic to nerve cells [6]. Furthermore, the accumulation of Aβ protein causes AD-associated events such as formation of NFT, cell loss, vascular damage, and dementia [4]. Based on the increased prevalence of early onset AD (EOAD) in Down syndrome patients with three copies of the APP gene and in people with the APP gain of function mutation that increases Aβ levels, Aβ has been thought to be a major cause of AD [7–9]. Supporting this idea, gain-of-function mutations of presenilin 1/2, a gene encoding the components of γ-secretase that processes APP to Aβ, which increased accumulation of Aβ, have been also associated with EOAD [9]. On the other hand, hyperphosphorylated tau, a microtubule-associated protein that stabilizes the microtubules, aggregates to form the NFT, resulting in neuronal degeneration [10]. Pathological tau aggregation is found in the brains of most AD patients, and the association between Aβ accumulation and tau phosphorylation has been reported [10]. Therefore, abnormal aggregation of tau protein is considered an important event in the pathogenesis of AD.
However, in the last decade, all clinical trials targeting Aβ or tau have failed, and the only available treatment remains symptomatic [11]. Therefore, despite decades of intensive research, the cause of AD remains elusive. In fact, many studies have demonstrated that AD is a complicated multifactorial disorder and may be affected by the combination of various genetic and environmental factors [12,13]. A twin study suggested that, depending on the model applied, the heritability of AD is 58% to 79% even though nongenetic risk factors also play an essential role [14]. Therefore, it is important to identify various genetic risk factors associated with AD for early detection and intervention. A series of genome-wide association studies (GWAS) have identified several genetic risk loci for late onset AD (LOAD), which seem to cluster in patterns that suggest immunity, lipid processing, and endocytosis as important causal biological processes [13]. In addition, the functional relevance of many AD-related factors has been demonstrated through functional genomic studies using genetic models, such as nematodes, fruit flies, and zebra fish, as well as mice [15].

1.2. Drosophila Models of Alzheimer’s Disease

Owing to the advantages of the Drosophila system, including the ability to withstand various genetic manipulations that cannot be performed in mammals, it has been an important model in AD studies [16,17] (Figure 1). There are three main types of Drosophila AD models according to the transgenes used, and the first type is the γ-secretase-based model. γ-secretase complex components are functionally conserved in the fly, and its many targets, such as APP and Notch receptor, are also conserved [18,19]. Overexpression of wild-type or familial AD-mutant presenilin (psn), a gene coding a component of γ-secretase complex, induces intracellular calcium deficits, which are regarded as one of the earliest events of AD pathology [20], whereas deficiency of psn causes associative learning defects and synaptic abnormalities in Drosophila larvae [21]. Thus, it follows, studies using γ-secretase-based AD models have facilitated understanding of the role of Presenilin in both development and degeneration as well as verifying many modifiers and pathways.

Furthermore, tau-based models have been established and used to study the role of tau in the formation of neurofibrillary tangles and neurotoxicity. For instance, several groups have shown that expression of human tau induces AD-like phenotypes in diverse Drosophila tissues [22,23]. A further study used a wild type or mutant human tau-expressing model to identify genetic modifiers of tau [24]. Moreover, the relationship between Aβ42 and tau has also been studied using Aβ42 and tau co-expression models [25].

Finally, most of the Drosophila AD models are based on APP or Aβ expression, since Aβ peptides, the major components of amyloid plaques, are considered to play the most important role in AD [26]. Because there is no conservation of both Aβ peptide sequence in APP and β-secretase in Drosophila, an essential condition for the generation of Aβ peptides, fly models expressing both human APP and BACE have been used [27–29]. In these models, AD-like phenotypes, such as age-dependent neuronal death, Aβ accumulation, and lethality are observed [27]. However, it has been found that APP-induced axonal defects are not caused by Aβ [30], and that the APP intracellular domain is involved in various processes such as axonal transport and synaptic plasticity [31]. Therefore, many groups have developed Drosophila AD models that directly express Aβ42 in the fly brain for a more direct study of the role of amyloid plaques in AD [32–35]. Each of the UAS-Aβ42 transgenes produced by these groups have differences in some part of the construct, such as the signal peptide, poly A tail, or the number of Aβ42 copies, which are directly related to the degree of Aβ peptide accumulation and intensity of AD-like phenotypes [36].

In this review, we will focus on the results obtained from models based on Aβ, the most commonly used AD models in Drosophila. The genetic modifiers found in studies using these Drosophila AD models to date suggest that several cellular pathways may be involved in the development of AD, and the results of these studies demonstrate the usefulness of the Drosophila model for finding related factors of multifactorial genetic diseases, such as AD.
2. AD-Related Mechanisms and Genetic Modifiers Identified Using the Drosophila Model

2.1. Amyloid Beta Accumulation

In the brain of Drosophila expressing Aβ42, age-dependent amyloid deposition was observed, as in human patient brains [37]. Moreover, ectopically expressed Aβ42 in Drosophila photoreceptors showed amyloidogenic and aggregating properties; the resistance to proteolytic cleavage, increased structural stability, and toxicity [32,35,38–40].

Recently, several studies showed the role of templated protein misfolding, referred to as seeding [41, 42], which induces misfolding and aggregation of the normal soluble protein [43]. Consistently, Drosophila models have provided evidence for a link between the seeding mechanism and neurotoxicity in vivo on a short time scale [44].

2.1.1. Soluble Aβ Oligomer Toxicity and Aggregation

Soluble Aβ oligomer was observed in the CSF of human AD [45] and was more closely associated with disease severity than amyloid plaque, insoluble Aβ, or fibrillar species [46]. Moreover, in other studies using ELISA and Western blotting, the amount of soluble oligomer was found to be more decisive for cognitive deficits than the simple plaque counts [47], and these soluble peptides induced progressive neuronal loss [48]. Consistently, Aβ peptide generation in the Drosophila retina shows age-dependent neurodegeneration in retinal photoreceptor cells and precedes the formation of Aβ plaques, suggesting that the Aβ oligomer and protofibril mediate toxicity [27]. The structural importance of Aβ to generate oligomer is also proved in Drosophila. A flavonoid derivative that interferes and disorders the Aβ oligomer and inhibitors targeting the α-helix of Aβ prevented...
Aβ-induced neurotoxicity in a Drosophila transgenic AD model [49,50]. A study showed the genetic interaction of neuroserpin, a natural inhibitor of tissue-type plasminogen activator that forms a binary complex with Aβ and prevents mature fibril formation of Aβ, with Aβ ectopically expressed in vivo in the Drosophila AD model [51]. Moreover, recent studies have shown that the cytosolic and secreted forms of the heat shock protein 70 (HSP70) prevent Aβ42 self-aggregation by binding to Aβ42, in which this reduction in aggregation by HSP70 significantly improved the memory performance of flies expressing Aβ42 [52,53]. In contrast, the mammalian prion protein stabilized Aβ oligomers and enhanced Aβ neurotoxicity in Drosophila [54]. Meanwhile, a recent study suggested a new hypothesis of a Aβ aggregation mechanism that gangliosides are responsible for Aβ assembly, by showing that ectopic expression of ganglioside synthesis enzymes in Drosophila, such as β1,4-galactosyltransferases (B4GalT6) and α2,3-sialyltransferase (SAT1), accelerate Aβ assembly [55].

2.1.2. Aβ Degradation

Since the accumulation of Aβ is critical in AD pathology, the Aβ catabolic pathway-related factors should be very important in the control of AD. The in vivo function of neprilysin (NEP) and insulin degradation enzyme (IDE) that is involved in the Aβ catabolic pathway were tested in the Drosophila AD model [56]. NEP belongs to the Aβ degrading enzymes, inhibition of which resulted in the pathologic deposition of Aβ in rats [56]. In the fly model, neuronal expression of human NEP led to significantly reduced intraneuronal deposits of Aβ42 in the brain and also suppressed Aβ42-induced neuron loss, suggesting that up-regulation of neuronal NEP activity is protective against intraneuronal Aβ42 accumulation and neuron loss [57]. IDE, a thiol metalloendopeptidase that cleaves small proteins, including insulin, has Aβ degrading activity [58], and IDE loss-of-function mutant mice showed elevated levels of neuronally secreted Aβ [58]. Consistent with mammals, the reduced lifespan of flies expressing APP and BACE in neurons was partially recovered by Drosophila Ide or human IDE expression, suggesting that IDE can inhibit the pathological processes associated with Aβ accumulation in vivo [59]. More recently, a study showed that partial knockout of neuronal Src homology 2B1 (SH2B1), an adaptor protein that is important for insulin receptor signaling, increased Aβ42 accumulation and had a detrimental effect on Aβ42-expressing flies, while overexpression of neuronal SH2B1 decreased Aβ42 accumulation and had beneficial effects [60]. These results suggested that the insulin signaling pathway plays important roles in Aβ metabolism.

The autophagic-lysosomal pathway is another important Aβ clearance pathway, the in vivo function of which in AD pathology is revealed in Drosophila. The hyperactivated PI3K/AKT/mTOR pathway, a negative-regulating pathway against autophagy, is linked to disrupted clearance of Aβ and tau [61] and alterations in this pathway are associated with autophagic dysfunction in the AD brain [62]. In Drosophila, genetic or pharmacologic inhibition of the PI3K/AKT/mTOR pathway improved Aβ-induced memory loss [63]. A recent study showed that ectopic expression of human Thioredoxin-80 (Trx80), a truncated form of Thioredoxin-1, prevents the toxic effects of Aβ and inhibits its aggregation [64]. In the same study, Gerenu and colleagues found that Trx80 exerts its protective activity through activation of autophagy. In addition, human phospholipase D3 (PLD3), a type-II transmembrane protein of the PLD family, exerts a neuroprotective effect against toxicity caused by Aβ when ectopically expressed in AD model flies, and the role of PLD3 in lysosome dynamics was considered to contribute to the beneficial effect of PLD3 [65]. Given the importance of autophagy in degenerative brain diseases, it is expected that more autophagy-related genes will be found as modifiers in AD pathology.

2.1.3. Intraneuronal Accumulation of Aβ

In addition to extracellular deposition, intraneuronal accumulation of Aβ has been revealed to be involved in pathological features of AD such as synaptic deficits, amyloid plaque formation, and cell death [66,67]. In the brains of AD patients, oligomeric Aβ is mainly localized in neurons, where it associates with lipid membranes [68]. As phosphoinositides, such as PI and PI4,5P, facilitate...
Aβ assembly in/on lipid membranes [69], their metabolizing enzymes affect AD pathogenesis by influencing neuronal accumulation of Aβ. Supporting this idea, a reduction in synaptotagmin-1, which converts PI4,5P into PI4P, inhibited synaptic and behavioral impairments in APP transgenic mice [70,71]. In *Drosophila*, the functions of the PI4KIIIα complex, which controls the levels of plasmalemmal PI4P and PI4,5P, are well conserved. Genetic reduction of components, such as PI4KIIIα, rolling blackout (RBO), tetratricopeptide repeat domain 7 (TTC7), and Hyccin, of the PI4KIIIα complex suppressed the phenotypes of Aβ42-expressing flies by reduction of neuronal Aβ accumulation [72,73]. In addition, another genetic modifier screening study identified *Drosophila* orthologues of human 4-hydroxyphenylpyruvate dioxygenase (HPD) and proline rich mitotic checkpoint control factor (PRCC) as suppressors of intraneuronal accumulation of Aβ [74]. Although HPD functions as 4-hydroxyphenylpyruvate dioxygenase that catalyzes the conversion of 4-hydroxyphenylpyruvate to homogentisate and PRCC may have a role in pre-mRNA splicing, the molecular mechanisms underlying how these proteins affect the intraneuronal accumulation of Aβ remain to be elucidated.

2.2. Amyloid-Mediated Tauopathy

Since phosphorylation of tau plays an important role in AD, there is a lot of interest in the regulators of tau phosphorylation. *Drosophila* has a tau homolog protein containing conserved disease-related phosphorylation sites of human tau [75]. Several studies have reported that Aβ42 induces phosphorylation and pathology of tau in flies and further noted that tau plays an important role in the downstream processes of Aβ-induced toxicity [25,76–78]. Aβ42 enhances tau-induced toxicity such as axonal transport defects, neuronal dysfunction, and reduced survival in Aβ42/tau co-expressing flies [25]. These effects also have been consistently observed in several studies using cell and rodent models [79–84]. In double-transgenic mice, hyperphosphorylated tau aggregation was decreased by clearance of Aβ, while Aβ accumulation was not affected by increasing tau [82].

Interestingly, it is known that par-1, a *Drosophila* orthologue of microtubule/microtubule-associated protein affinity regulating kinase (MARK), and glycogen synthase kinase 3β (GSK3β), a component of the Wnt pathway, are critical for Aβ-induced tau phosphorylation in the fly model [25,78]. Several in vitro studies support the idea that Aβ promotes tau phosphorylation via GSK3β activity [85–88], and studies using mouse models indicate that MARK phosphorylates tau [89,90]. In human cell models, more than 40 tau phosphorylation sites are associated with AD [91–94]: Serine (Ser) 262 and Ser356 are phosphorylated by MARK [89,90], and Threonine (Thr) 231, Ser199, Ser202, Ser235, Ser396, and Ser404 by GSK3β [95–98]. In *Drosophila*, par-1 and GSK3β can also phosphorylate most of these phosphorylation sites of tau [99], suggesting that the catalytic function of the two kinases is conserved between insects and humans. Furthermore, it was first observed in flies that phosphorylation of tau by par-1 plays an important role in the subsequent phosphorylation of tau by GSK3β, suggesting that tau phosphorylation happens in a structurally arranged pattern [99,100]. When Ser262 and Ser356 in tau are substituted for non-phosphorylatable Ala, Aβ42-mediated tau phosphorylation at Thr231 by GSK3β is blocked [78].

In mice, Aβ42 toxicity occurs through Aβ-induced phosphorylation of tau, and reduction or clearance of tau alleviates phenotypes and toxicity of Aβ42; for instance, memory impairment, synaptic loss, neuron loss, and premature death [83,101]. Furthermore, a study showed that removal of endogenous *Drosophila* tau reduces Aβ42-induced locomotor dysfunction in flies [102]. However, another recent study revealed that deletion of endogenous *Drosophila* tau had no effect on Aβ42-induced premature death in the fly model [103]. Therefore, unlike in mammals, it is controversial whether endogenous *Drosophila* tau contributes to Aβ42-induced toxicity.
2.3. Modifiers Related to Stress-Responsive Pathways

2.3.1. Oxidative Stress

It has been known that Aβ42 causes oxidative stress, which is believed to be augmented in the brain of AD patients and animal models [104–106]. Based on the important role of oxidative stress in AD pathology, the interest in genes associated with oxidative stress has increased. Although several groups have shown that these genes affect AD pathology through screening studies in Drosophila [107–111], the effects of antioxidant genes, such as superoxide dismutase (SOD), on AD remain controversial.

Rival and colleagues carried out unbiased screening to identify genes whose expressions were changed by Aβ42 and found that antioxidative stress genes, such as Ferritin 1 heavy chain homolog (Fer1HCH), Ferritin 2 light chain homolog (Fer2LCH), catalase (CAT), and Sod2, reduced Aβ42-induced toxicity [107]. In other studies, Fer1HCH and Fer2LCH inhibited Aβ42-induced eye phenotype and premature death [108], while knockdown of Fer1HCH and Fer2LCH increased Aβ42-induced toxicity [109]. In addition, overexpression of sarah (sra), a Drosophila orthologue of protein-serine/threonine phosphatase regulator Down Syndrome Critical Region 1, increases the hydrogen peroxide susceptibility and enhances Aβ42-induced toxicity as a result of reducing the expression of Sod2, Sod3, and Glutathione S transferase D1 in Aβ42/sra-coexpressing flies [111].

In contrast, Favrin and colleagues identified 712 genes of whose expressions were changed in Aβ42-expressing flies and demonstrated that knockdown of Sod3, an extracellular superoxide dismutase gene whose expression increases in Aβ42-expressing flies, increases the locomotor defect and premature death in AD model flies [112]. Expression of wild type Sod1, another ROS-associated gene, decreases the lifespan of Aβ42-expressing flies, while a dominant negative form of Sod1 rescues premature death of the AD model flies [107]. These detrimental effects of Sods on Aβ42-expressing flies may be due to the toxic hydrogen peroxide overload, which occurs because of an imbalance between Sod, which produces hydrogen peroxide, and CAT, which catalyzes the decomposition of hydrogen peroxide to water and oxygen. Furthermore, the functional differences between these SODs may be also due to the distinct locations and prosthetic groups of the enzymes: Sod1 and Sod3 is Cu/ZnSOD in the cytoplasm and extracellular space, respectively, and Sod2 is MnSOD in the mitochondria [113, 114]. Therefore, it is possible that there is a difference in ROS function between the mitochondria and cytoplasm in AD pathology.

It is believed that factors related with metals also play important roles in the cellular response to oxidative stress [115]. ROS generated by Aβ42 damages the cellular membrane, especially in the presence of metals such as copper, zinc, and iron [115]. Intake of copper or zinc enhances Aβ42 toxicity, while genetic inhibition of copper transporter 1B (Ctr1B) or Ctr1C, copper importers, or zinc/iron regulated transporter-related protein 1 (Zip1), a zinc importer, alleviates premature death and locomotor defects in AD model flies [116,117].

AD is strongly associated with oxidative stress, and many oxidative stress-related genes, including metals- and mitochondria-related genes, affect AD pathology. Although many antioxidant genes have been shown to have beneficial effects on AD, the protective effect of SOD remains controversial and further studies should be conducted.

2.3.2. Endoplasmic Reticulum Stress

Endoplasmic reticulum (ER) stress has also been implicated in AD [118] and can be induced by Aβ42 in cultured cells, mice, and flies [35,119–122]. During the course of ER stress, a fragment of activating transcription factor 6 (ATF6) moves into the nucleus and expresses ER stress response genes, including X-box binding protein 1 (XBP1) [123]. Expression of XBP1 is also promoted by Aβ42, and overexpression of spliced XBP1 (XBP1-S), the activated form of XBP1, reduces Aβ42 toxicity in Drosophila photoreceptors, whereas knockdown of endogenous XBP1 intensifies rough eye phenotype induced by Aβ42 [35]. Moreover, a recent study showed that overexpression of XBP1-S in the fly brain reduces Aβ42 levels and improves Aβ42-induced locomotor dysfunction,
while reduction of endogenous XBP1 increases Aβ42 protein levels and enhances Aβ42-induced locomotor dysfunction [124]. Furthermore, chronic expression of Aβ42 activates protein kinase R-like endoplasmic reticulum kinase (PERK) and ATF6 pathways, both major branches of the unfolded protein response, as well as inositol-requiring enzyme 1 α-XBP1 pathway, and Aβ42-induced activation of PERK may have a beneficial effect on AD by Aβ42 clearance [124]. Therefore, Drosophila studies suggest that the ER stress response pathways may be implicated in the pathogenesis of AD.

2.4. Modifiers Involved in ERK Pathway or Cell Cycle

2.4.1. EGFR/ERK Signaling

Activation of the extracellular signal-regulated kinase (ERK) pathway, as well as other mitogen-activated protein kinases (MAPKs) that include c-jun N-terminal kinase (JNK) and p38 MAPK, has been observed in AD neurons and animal models [125–128]. The ERK pathway has been reported to play a crucial role in dead signaling in neurons [129–131], although it is commonly thought to be a survival signal [132,133]. In Drosophila, Aβ42 activates ERK, and pharmacological inhibition of ERK activity reduces neurotoxicity of Aβ42, suggesting that chronic activation of ERK is a crucial step in the progression of AD [131]. Furthermore, extracts of Chinese medical herbs, such as C. sativum and N. jatamansi that inhibit ERK activation, ameliorate AD phenotypes in cultured mammalian cells and flies [134,135]. Epidermal growth factor receptor (EGFR) signaling within a particular range is necessary to maintain homeostasis of mushroom bodies, which is required for neuronal plasticity, learning, and memory [136]. However, excessive EGFR aggravates short-term memory loss of Aβ42-expressing flies, while treatment with gefitinib or erlotinib, two EGFR inhibitors, suppresses Aβ42-induced memory loss [137].

2.4.2. Cell Cycle

Erroneous cell cycle re-entry (CCR) in neurons has been considered to be a crucial causative factor in neuronal death [138]. In AD brains, vulnerable neurons show activated cell cycle markers and re-expression of cell cycle regulators. Yet they are incapable of completing the cell cycle, resulting to the aberrant neuronal death [139,140]. The Aβ42 oligomer induces CCR in cultured primary neurons, and neuronal CCR takes place before accumulation of Aβ42 in APP-expressing rat brains [141,142]. Consistently, in the Drosophila brain, Aβ42 increases expression of Cyclin B, an important cell cycle protein, while genetic reduction of Cyclin B extends the lifespan and improves locomotor dysfunction of AD flies [143]. APP also induces erroneous CCR, and knockdown of polo, another key regulator of the cell cycle, partially rescues APP-induced locomotor dysfunction and retinal degeneration and prevents a shortened lifespan by repressing APP-induced CCR [144].

It is also known that Notch activation, which is essential for neuronal specification and development, is implicated in erroneous CCR and AD [145–147]. In the rodent model, kainic acid-induced activation of Notch results in neurodegeneration through erroneous CCR [146]. Moreover, genetic reduction of Delta, a ligand of Notch, and N-[N-(3,5-Difluorophen-acetyl)-L-alanyl]-S-phenylglycine t-butyl ester, a Notch inhibitor, rescued learning impairments and prevented the premature death of Aβ42-expressing flies [147].

2.5. Modifiers Related to Apoptosis

Apoptosis is a major pathway of neurodegenerative cell death in AD, and several apoptosis-related factors have been identified as genetic modifiers of AD pathology [23,148]. In Drosophila, apoptotic cell death induced by ectopic expression of Aβ42 was detected by several methods, including active caspase 3 antibody staining, TUNEL assay, and acridine orange staining in the fly brain [53,149]. Moreover, several studies have shown that anti-apoptotic proteins have protective effects on AD-like phenotypes in Aβ42- or tau-expressing flies [24,38,150]. For example, co-overexpression of baculovirus p35,
a caspase inhibitor, partially inhibits Aβ42-induced cell death in fly photoreceptors [38], implying that Aβ42-mediated cell death occurs via both p35-sensitive caspase-dependent and -independent pathways. In contrast, another study showed that death-associated inhibitor of apoptosis 1 (DIAP1), a Drosophila homolog of the inhibitor of apoptosis proteins, almost completely suppressed the AD-like phenotype, including Aβ42-induced cell death in the fly brain [150]. Given that Dronc, an initiator of caspase, can be inhibited by DIAP1 but not by p35 [151–153], the difference in the degree of protective effects between p35 and DIAP1 suggests the role of Dronc in Aβ42-induced cell death. In addition, deficiency of the translocator protein 18 kDa (TSPO), an outer mitochondrial membrane protein that plays an important role in the regulation of apoptosis, rescues the reduced lifespan of Aβ42-expressing flies by decreasing apoptosis and caspase 3 and 7 activities [154].

JNK and the stress-activated protein kinase subfamily have been also implicated in AD pathology [155–158]. JNK signaling is up-regulated by Aβ42 and also contributes to Aβ42-induced cell death in Drosophila [38,150]. Furthermore, inhibition of JNK through genetic modification or pharmacological treatment alleviates Aβ42-induced neuronal cell death, reduced survival rate, and locomotor dysfunction in flies [150]. Additionally, night-time sleep loss of Aβ42-expressing flies is restored by JNK inhibition [159].

The detrimental effect of JNK in AD model flies has been also revealed through the modulation of JNK upstream and downstream factors [24,150,160,161]. Hemipterous (hep), a Drosophila homolog of the JNK kinase, enhances Tau-induced toxicity in fly eyes [24], while hep deficiency reduces neuronal cell death of Aβ42-expressing larvae [160]. Additionally, a deficiency of Drosophila forkinghead box subgroup O (dFOXO), a downstream factor of JNK, suppressed Aβ42-induced neuronal cell death, the reduced survival rate, and locomotor dysfunction of Aβ42-expressing flies [150]. Expression of human amyloid precursor like protein-1 (APLP1), a component of the amyloid precursor protein family, in flies increases transcription of dFOXO target pro-apoptotic genes, hid and reaper, and APLP1-induced cell death is rescued by genetic reduction of dFOXO [161].

A recent study has highlighted another aspect of neuronal cell death in the brain of AD model flies [162]. Apoptosis promotes the elimination of impaired neurons from brain circuits, protecting the brain instead of attacking it. In the same study, Coelho and colleagues demonstrated that suppression of fitness-based removal of Aβ42-damaged neurons by knockdown of azot, the fitness checkpoint gene, and overexpression of DIAP1 exacerbated AD-like phenotypes of Aβ42-expressing flies, including degenerative vacuoles, a decreased lifespan, a locomotory defect, and a memory defect. This new finding shows that the role of neuronal cell death in the AD brain is more complex than previously thought.

2.6. Modifiers Related to Epigenetic Regulation

Based on its important function in neurons, epigenetic mechanisms have been suggested to play a pivotal role in AD pathophysiology [163]. Histone acetylation, which is regulated by the activities of histone deacetylases (HDAC) and histone acetyltransferases (HAT), has been primarily implicated in AD-like phenotypes in Drosophila. HDAC6 is a unique member of the HDAC family that acts mainly on cytoplasmic non-histone substrates [164] and increased in a postmortem study of human AD brain [165]. A study of mammals showed that Aβ-induced mitochondrial transport was rescued by inhibition of HDAC6 [166]. In Drosophila, microtubule defects in human tau-expressing flies were rescued by HDAC6 inhibition [167]. However, another study indicated that histone deacetylase inhibitor trichostatin A caused lethality and delayed development in Drosophila [168], as well as inducing neuronal death in mice [169].

γ-cleavage of APP releases an intracellular tail that forms a complex with Tip60, a member of the HAT family, which affects the pathophysiology of AD [170]. In APP-overexpressing transgenic mice, levels of Tip60 are increased [171]. In contrast, in Drosophila, Tip60 levels are decreased, while HDAC1 levels are increased, in the larval brain of APP-overexpressing flies, which resulted in epigenetic repression of neuroplasticity genes [172]. Moreover, specific loss of Tip60 activity enhanced
APP-mediated lethality and neuronal apoptotic cell death in *Drosophila*, while overexpression of Tip60 diminished these defects and improved the learning and memory performance of APP-expressing larvae [172,173]. Another HAT family protein, the CREB-binding protein (CBP), has also been reported to play a neuroprotective role in the *Drosophila* AD model. The expression of CBP in *Drosophila* expressing Aβ42 in the retina rescued eye phenotype, apoptosis, neurodegeneration, and axonal targeting defects. This neuroprotective effect of CBP was found to be essential for the bromo, HAT, and poly glutamine stretch (BHQ) domain of CBP [174].

### 2.7. Modifiers Related With Synaptic Abnormalities

Failure of normal synaptic function might be one of the earliest measurable deficits in AD [175], and the decreases in synaptic density appear to occur early in the course of AD in mouse models and patients [176–179].

Aβ peptides have been suggested to have physiological roles in synaptic function [180], and Aβ oligomers specifically target molecular components that mediate synaptic plasticity [181]. In several studies, mutant APP transgenic mice showed synaptic dysfunction before plaque formation, suggesting that soluble Aβ levels in the cortex significantly correlate with the degree of synaptic loss [178,182–184]. Studies in *Drosophila* have shown that early memory defects and structural and/or functional synaptic defects are induced by expression of Aβ, and that Aβ peptides inhibit the formation and/or maturation of new synapses [37,63,185,186]. Thus, antibodies to neutralize soluble Aβ oligomers are suggested as treatment for early AD [187]. Consistent with these findings, various factors that act on normal synaptic function have been found to be AD-related factors, as follows.

#### 2.7.1. Small GTPases

Small GTPases, such as Rho and Rac1, play a prominent role in the development of dendritic structure [188]. For example, the constitutive active (CA) form of RhoA reduces dendritic arbor growth in *Drosophila* [189], and CA Rac1 tends to form ruffle-like structure in rodent neurons [190].

Several studies implicated these small GTPases in AD pathology. It has been shown that RhoA expression was decreased in synapses and increased in dystrophic neurites in a mouse AD model, suggesting that RhoA might be associated with AD pathology [191]. In *Drosophila*, increased activation of Rho1, the *Drosophila* orthologue of vertebrate RhoA, by prenylation, a posttranslational modification facilitating the association of proteins with membranes, leads to age-dependent degeneration of the nervous system [192]. A recent study demonstrated that Rac1 activity was abnormally increased in the hippocampal tissues of AD patients and mouse AD models, and that inhibition of the elevated Rac1 activity rescued memory loss in both fly and mouse AD models [193].

#### 2.7.2. Impaired Axonal Transport

Impaired transportation in neurons is regarded as an underlying cause of synaptic failure in AD [194], and overexpression of APP leads to axonal transport defects in fly and mouse models [195–197]. In the peripheral nerves of *Drosophila* larvae expressing APP, a “traffic jam” of vesicles was observed, and expression of scaffolding proteins, Fe65 and JIP1b, which interact with the APP intracellular domain, also induced the axonal transport defect [198].

#### 2.7.3. Synaptic Proteins

It has been shown that the expression levels of genes involved in synaptic vesicle trafficking are decreased in the brain of AD patients [199]. In flies, expression of Bruchpilot, a homolog of ELKS/RAB6-interacting/CAST family member 2 (ERC2) that can affect the localization of Ca2+ channels in presynaptic release sites [200], was not significantly different in APP- and BACE-expressing larvae compared to the control group [201] and five-day-old Aβ-expressing flies, but showed reduced levels at 21 days of age [202]. Additionally, the levels of Discs-large, a homolog of DLG4 essential for the density of the synaptic glutamate receptor [203,204], were significantly reduced in fly larvae.
expressing APP and BACE [201]. A recent study proved the importance of the synaptic localization of a subset of synaptic proteins, including APP [205]. In this study, Furotani and colleagues demonstrated that knockdown of yata, a novel gene regulating the synaptic localization of β amyloid protein precursor-like and other synaptic proteins, rescued the phenotypes of APP-expressing flies, suggesting that the regulators of the synaptic localization of synaptic proteins are involved in AD pathology.

2.8. Mitochondrial Dysfunction or Mislocation

Mitochondrial dysfunction has been reported to contribute to the progression of AD pathology [206]. For example, Dynamin-related protein 1 (Drp1), a key regulator of mitochondrial fission, exerts a beneficial effect in Aβ42-expressing flies by protecting mitochondria [207]. In contrast, sra overexpression reduced the number of mitochondria and enhanced Aβ42 toxicity [111]. Also, the mislocation of mitochondria has been implicated in AD pathology. Mitochondria are dynamic organelles whose active movement is essential for the mobilization of the synaptic vesicle reserve pool [208]. In the brains of AD patients and the AD model mouse, disruption of mitochondrial transport resulted in the reduced distribution of mitochondria in the axon and dendrites [196]. Furthermore, mislocalization of mitochondria was observed in the brain of flies expressing Aβ42 [209]. Therefore, restoration of mitochondrial transportation could be a promising target mechanism that can be used to develop AD therapies.

2.9. Modifiers Related with Inflammation

The immune system has been known to be aberrantly activated in the brains of AD patients and contributes to AD pathogenesis [210,211]. As the immune response not only provides a protective role by promoting the clearance of toxic Aβ aggregates but also exerts a detrimental effect on AD pathology by up-regulating chronic inflammation, it has been regarded as a double-edged sword in neurodegenerative disease [212]. In particular, innate immunity and neuroinflammation are thought to be essential components of neurodegeneration in both fly and mammalian brains [213–215].

2.9.1. Phagocytic Receptor Draper

After Aβ is produced in the CNS, binding of soluble Aβ oligomer and fibrils to the microglial receptor produces a signal that results in the production of cytokines and chemokines [216]. In Drosophila, glial cells show functional and morphological similarity to those of mammals [217] and contribute to the protection of neurons, engulfing Aβ fibrils by phagocytosis in the fly AD model [218]. Glial phagocytosis may decrease with aging due to decreased levels of Draper, a Drosophila homolog of the mammalian Multiple EGF Like Domains 10, which is a conserved phagocytic receptor of glial cells [219], and up-regulation of Draper which can reverse the Aβ-related phenotype [218], suggesting that the phagocytic function of glial cells may exert a beneficial effect in neurodegenerative disease.

2.9.2. TREM2

Triggering Receptor Expressed on Myeloid cell 2 (TREM2) is another receptor that mediates phagocytosis on the microglial surface [220] and regulates inflammatory responses via Toll-like receptors (TLRs) in AD [221,222]. TREM2 suppresses inflammation through the inhibition of cytokine production [223] and has a protective effect in AD by reducing inflammation-induced neuronal damage [224]. TREM2 mutation correlates with a significantly increased risk of AD [225], and TREM2 deficiency promotes Aβ accumulation due to a dysfunctional microglial response [226]. There is no apparent Drosophila homolog of TREM2. However, a recent study using Drosophila AD models shows that glial expression of human TREM2 or human tyrosine kinase binding protein (TYROBP), the intracellular adaptor of TREM2, did not affect AD-like phenotypes of Aβ42-expressing flies, while glial expression of TREM2/TYROBP modifies molecular signatures induced by neuronal expression of Aβ42 [227]. Unlike Aβ42, TREM2/TYROBP expression in glial cells exacerbated
tau-mediated AD pathology [227]. Therefore, further detailed research using various AD models, on the functions of TREM2 in AD pathology is needed.

2.9.3. Toll and IMD Pathways

Toll and immune deficiency (IMD) pathways are the major innate immune responsive pathways in Drosophila that regulate the production of antimicrobial peptides [228]. The Toll pathway that is mainly responsive to fungi, Gram-positive bacteria, and virulence factors, functions through the NF-kB family transcription factors, Dorsal and Dif, while the IMD pathway, responsive to Gram-negative bacteria, acts through another NF-kB family transcription factor, Relish.

In mammals, several TLRs are involved in \( A\beta \) uptake by microglial cells and activate innate immune responses to prevent \( A\beta \) accumulation in the CNS [221,229,230]. In AD brains, high expression of TLR was detected [231], and TLR-deficient mice showed increased \( A\beta \) deposits [221], suggesting that TLR is involved in \( A\beta \) clearance. However, loss-of-function mutations of Drosophila Toll (Tl) suppresses the pathological effects of human \( A\beta 42 \) in the Drosophila AD model [232]. Moreover, the same study showed that deficiencies in the downstream components of the Toll pathway, including an adaptor protein Tube, the IRAK-like kinase Pelle, and Dorsal and Dif, ameliorated the rough eye phenotype of human \( A\beta 42 \)-expressing flies [232]. The difference between the mammalian and fruit fly results probably reflects the dual aspect of the Toll pathway, i.e., clearance and inflammation.

The IMD pathway has also been implicated in the toxicity of \( A\beta 42 \) in Drosophila. The expression of peptidoglycan recognition protein SC1b (PGRP-SC1b), a suppressor of the IMD pathway, is up-regulated in \( A\beta 42 \)-expressing flies, and knockdown of PGRP-SC1b ameliorated the reduced lifespan and locomotory defect of the fly AD model [112], suggesting that the IMD pathway is protective against \( A\beta 42 \) toxicity. In contrast, a recent study showed that the downregulation of Relish, a downstream transcription factor of the IMD pathway, in Drosophila astrocytes ameliorated the toxicity of \( A\beta 42 \) as well as polyglutamine [233]. These confusing findings highlight the complexity of the role of the immune response in pathogenesis of AD.

3. Conclusions and Perspectives

In this study, we have reviewed the AD-associated genes found in the Drosophila models and their cellular pathways (Table 1 and Figure 2). Because AD is a multifactorial disease, various AD-related genetic factors and their functions need to be identified before developing accurate tests and treatments for this disease. Therefore, genetic studies focusing on the discovery of various AD-related genes are considered essential for identifying the causes of AD and developing therapies. With the recent application of GWAS and next-generation sequencing, 29 LOAD-related factors have been discovered, and research into the functions of these factors will broaden our understanding of AD etiology [234,235]. However, despite intensive research over the past decades, our understanding of the AD-related genes discovered to date is limited. In fact, a study showed that known AD-risk variants can explain only about 30% of AD variance, indicating that many genetic loci remain to be discovered [236]. Therefore, new approaches may be needed to boost the probability of identifying causal genes and pathways.

Functional genomic studies using Drosophila could be an alternative approach to finding new AD-related genes. The results of studies with Drosophila AD models described in this study are sufficient to show the feasibility of using Drosophila models to find new disease modifiers. While there are drawbacks—the Drosophila models are over simplified and have relatively low relevance to humans compared to mice—the advantages of using Drosophila models outweigh these drawbacks. Specifically, the Drosophila models have a wide variety of genetic tools, and it takes less than 10 days for the AD phenotype to appear, whereas it takes more than six months in the mouse AD model, although it varies from model to model [237], and allows for large-scale screening. In addition, the presence of RNAi for all genes annotated in its genome and simple crosses allow the screening of genetic modifiers for the toxicity of \( A\beta 42 \) and tau. Therefore, more AD-related genes are expected to be discovered in the future thanks to Drosophila models. In particular, if there are limitations in the discovery of
disease-related genes because of the small sample size in human GWAS studies, it will be possible to discover new genes by conducting a combined study of GWAS and *Drosophila* screening. *Drosophila* models will also be a good tool for in vivo functional studies of the genes discovered via human genetic studies. In the future, this combination of human genetics and *Drosophila* functional genomics is expected to be an important strategy for research into multifactorial diseases, such as AD.

### Table 1. Genetic modifiers of amyloid precursor protein (APP) or Aβ-based *Drosophila* Alzheimer’s disease (AD) models.

| Pathway                  | *Drosophila* Genes | Human Genes | Protein Type and Function                                   | Effect | References       |
|--------------------------|--------------------|-------------|-------------------------------------------------------------|--------|------------------|
| Aβ production and aggregation | Spn42Da          | SERPIN1     | Serine protease inhibitor that interacts with tissue plasminogen activator | S      | [51]             |
|                          | Hsp70              | HSPOA1A      | Central component of the cellular network of molecular chaperones and folding catalysts | S      | [52,53]         |
|                          | 4GaINAcTA          | BAGALT6      | β-1,4-galactosyltransferase                                  | E      | [55]             |
|                          | CG4210             | SAT1        | α-2,3-sialyltransferase                                      | E      | [55]             |
|                          | Ncp1               | MME         | Membrane metalloendopeptidase                                | S      | [57]             |
|                          | Id8                 | IDE         | Zinc metalloendopeptidase that degrades insulin              | S      | [59]             |

| Aβ production and aggregation | LinkSH2B           | SH2B1       | SH2-domain containing mediator important for insulin receptor signaling | S      | [60]             |
| PIIK21B                   | PIK3R3             |             | Regulator subunit of PI3K that phosphorylates phosphatidylinositol | E      | [63]             |
| Akt                       | AKT                |             | AKT serine/threonine kinase                                  | E      | [63]             |
| Ter                       | MTO8               |             | Phosphatidylinositol kinase-related kinase                   | E      | [63]             |
| Pld3                      | PLD3               |             | Enzyme that catalyzes the hydrolysis of membrane phospholipids | S      | [65]             |
| PIKIIa                    | PI4KA              |             | Lipid kinase that synthesizes phosphatidylinositol 4-phosphate from phosphatidylinositol | E      | [72]             |
| RBOIstmA                  | EFR3B              |             | Component of complex required to localize PI4K to the plasma membrane | E      | [72]             |
| Tc7                       | TTC7R              |             | Component of complex required to localize PI4K to the plasma membrane | E      | [73]             |
| Hycin                     | FAM126A            |             | Component of complex required to localize PI4K to the plasma membrane | E      | [73]             |
| Hpyd                      | HPD                |             | 4-hydroxyphenylpyruvate dioxygenase that catalyzes the conversion of 4-hydroxyphenyl-pyruvate to homogentisate | S      | [74]             |
| CG17249                   | PRCC               |             | Protein that may play a role in pre-mRNA splicing            | S      | [74]             |

| Tauopathy                 | par-1              | MARK        | Serine/threonine kinase that plays critical roles in cell polarity and microtubule dynamics | E      | [99]             |
|                          | gsk3β              | GSK3β       | Serine/threonine kinase that plays multiple roles in various signaling pathways | E      | [99]             |

| Oxidative stress          | Fer1HCH            | FTH1        | Subunit of Ferritin, an iron-storage protein                 | S      | [107–109]       |
|                          | Fer2LCH            | FTM1        | Subunit of mitochondrial Ferritin, an iron-storage protein   | S      | [107–109]       |
|                          | Cat                | CAT         | Enzyme that catalyzes decomposition of hydrogen peroxide to water and oxygen | S      | [107]           |
|                          | Sod2               | SOD2        | Mitochondrial Mn-dependent superoxide dismutase              | S      | [107]           |
|                          | sra                 | RCAN1       | Inhibitor of calcineurin                                      | E      | [111]           |
|                          | Sod3               | SOD3        | Extracellular Cu/Zn-dependent superoxide dismutase           | E      | [112]           |
|                          | Sod1               | SOD1        | Cytoplasmic Cu/Zn-dependent superoxide dismutase             | E      | [107]           |
|                          | Ctr1B              | SLC31A1     | Copper importer                                              | E      | [117]           |
|                          | Ctr1C              | SLC31A1     | Copper importer                                              | E      | [117]           |
|                          | Zip1               | SLC39A3     | Zinc importer                                                | E      | [116]           |

| ER stress                 | Xbp1               | XBP1        | Transcriptional factor that mediates the unfolded protein response | S      | [35,124]       |
|                          | PERK               | EIF2AK3     | ER transmembrane kinase that phosphorylates eukaryotic translation-initiation factor 2-alpha during ER stress | S      | [124]           |

| EGFR/ERK pathway          | rl                 | MAPK4       | Serine/threonine kinase and core component of the EGFR/MAPK pathway | E      | [131]           |
|                          | Egfr               | EGFR        | Membrane-localized tyrosine kinase receptor for epidermal growth factor | E      | [137]           |

| Cell cycle                | CycB               | CCNB1       | C2/mitotic-specific protein which is a member of the cyclin family | E      | [143]           |
|                          | polo               | PLK1        | Serine/threonine kinase involved in mitosis                   | E      | [144]           |
|                          | N                  | NOTCH1      | Transmembrane receptor for Notch signaling                     | E      | [147]           |
|                          | DI                 | DL1         | Transmembrane ligand for Notch signaling                       | E      | [147]           |
In addition, Drosophila can also be used as a screening tool for drugs to modify AD symptoms or disease progression. Unfortunately, all recently developed AD treatment candidates have failed in the clinic and thus, the causative factors for the disease have come into question [238,239]. Despite such factors, AD progression, including accumulation of Aβ in the brain, begins long before the appearance of clinical symptoms such as memory loss, and most AD patients are diagnosed at a later stage of neurodegeneration. Recently, the FDA recognized this characteristic and issued an amendment to permit clinical trials very early in the disease [240]. As a result, it is anticipated that clinical trials of new drugs for these early diagnosis and early stage patients will increase rapidly. Therefore, finding more drug candidates will be another important task in this field.

Drosophila is a well-characterized insect with various phenotypes and is a model system that can be used to rapidly screen many drug candidates in vivo. Indeed, many studies to date have used the Drosophila AD model to screen for small molecules that can modify AD symptoms or validate the in vivo efficacy of developed drug candidates [29,241–243]. In addition, Drosophila has been used to screen natural products and traditional medicines that are expected to have therapeutic and prophylactic properties in AD [244,245]. In the future, the superiority of the Drosophila model as an in vivo drug screening system is expected to increase its utility.

### Table 1. Cont.

| Pathway                  | Drosophila Genes | Human Genes | Protein Type and Function                                      | Effect | References |
|--------------------------|------------------|-------------|----------------------------------------------------------------|--------|------------|
| Apoptosis                | Diap1            | BIRC2       | E3 ligase with inhibitory activity on caspase                  | S      | [150]      |
|                          | Tspo             | TSPO        | Outer mitochondrial membrane protein related to steroid         | E      | [162]      |
|                          | bzk              | MAPK8       | Serine/threonine kinase that phosphorylates the Jra transcription factor | E      | [38]       |
|                          | hep              | MAP2K7      | Serine/threonine kinase involved in the JNK pathway by          | E      | [150]      |
|                          | foxo             | FOXO        | Forkhead family of transcription factor regulated by various    | E      | [150]      |
|                          | azot             | CALM1       | EF-hand calcium binding protein act as fitness checkpoint       | S      | [162]      |
| Epigenetic regulation    | Type60           | KAT5        | Histone acetyltransferase                                       | S      | [172]      |
|                          | nei              | CBP         | Histone acetyltransferase                                       | S      | [174]      |
| Synaptic abnormalities   | Rac1             | RAC1        | GTPase which belongs to the RAS superfamily of small GTP-binding proteins | E      | [193]      |
|                          | Bruchpilot       | ERC2        | Cytoskeletal protein critical for structural integrity of        | S      | [202]      |
|                          | yata             | SCY1        | Transcriptional regulator belonging to the SCY1-like family of  | E      | [205]      |
| Mitochondrial dysfunction or mislocation | Drp1            | DNML4       | Dynamin related protein that regulates mitochondrial fission   | S      | [207]      |
|                          | sra              | RCAN1       | Inhibitor of calcineurin                                        | S      | [197]      |
|                          |                  |             |                                                                |        | [111]      |
| Inflammation and innate immune system | Draper          | MEGF10      | Multiple EGF like domains 10, which encodes a phagocytic receptor of glia | S      | [218]      |
|                          | Ti               | TLR         | Toll-like receptor that promotes NF-kB like transcription factors | E      | [232]      |
|                          | tub              | IRAK1       | Downstream component of the Toll pathway                        | E      | [232]      |
|                          | phi              | IRAK4       | Downstream component of the Toll pathway                        | E      | [232]      |
|                          | dl               | RELA        | Transcription factor regulated by the Toll pathway              | E      | [232]      |
|                          | Dif              | RELB        | Transcription factor regulated by the Toll pathway              | E      | [232]      |
|                          | PGRP-SC1b        | PGLYRP1     | Peptidoglycan recognition protein SC1b which is a negative regulator of Imd pathway | E      | [112]      |
|                          | Rel              | NFκB1       | Subunit 1 of nuclear factor kappa B, which is a main regulatory gene in the Imd pathway | E      | [233]      |

1 Suppressor; 2 Enhancer.
Figure 2. AD-related mechanisms identified using the APP- or Aβ-based Drosophila model. Ac, acetylation; CCR, cell cycle re-entry; ER, endoplasmic reticulum; ROS, reactive oxygen species.

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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AD | Alzheimer’s disease |
| Aβ | Amyloid β |
| APLP1 | Amyloid precursor like protein 1 |
| APP | Amyloid precursor protein |
| ATF6 | Activating transcription factor 6 |
| B4GALT6 | β1,4-galactosyltransferases |
| BHQ | Bromo, HAT and poly glutamine stretch |
| CA | Constitutive active |
| CAT | Catalase |
| CCR | Cell cycle reentry |
| CBP | CREB-binding protein |
Ctr1B  Copper transporter 1B
DFOXO  Drosophila forkhead box subgroup O
DIAP1  Death-associated inhibitor of apoptosis 1
Drp1  Dynamin-related protein 1
EGFR  Epidermal growth factor receptor
EOAD  Early onset AD
ER  Endoplasmic reticulum
ER2C  ELKS/RAB6-interacting/CAST family member 2
ERK  Extracellular signal-regulated kinase
Fer1HCH  Ferritin 1 heavy chain homologue
Fer2LCH  Ferritin 2 light chain homologue
GSK3β  Glycogen synthase kinase 3β
GWAS  Genome-wide association studies
HAT  Histone acetyltransferases
HDAC  Histone deacetylases
hep  hemipterous
HPD  4-hydroxyphenylpyruvate dioxygenase
Hsp70  Heat shock protein 70
IDE  Insulin degradation enzyme
IMD  Immune deficiency
JNK  c-jun N-terminal kinase
LOAD  Late onset AD
MARK  Microtubule affinity regulating kinase
MAPKs  Mitogen-activated protein kinases
NEP  Neprilysin
NFTs  Neurofibrillary tangles
PERK  Protein kinase R-like endoplasmic reticulum kinase
PGRP-SC1b  Peptidoglycan recognition protein SC1b
PLD3  Phospholipase D3
PRCC  Proline rich mitotic checkpoint control factor
psn  presenilin
RBO  Rolling blackout
SAT1  α,2,3-sialyltransferase
Ser  Serine
SH2B1  Src homology 2B1
SOD  Superoxide dismutase
sra  sarah
Thr  Threonine
TLRs  Toll-like receptors
TREM2  Triggering receptor expressed on myeloid cell 2
Trx80  Thioredoxin-80
TSPO  Translocator protein 18 kDa
TTC7  Tetratricopeptide repeat domain 7
XBP1  X-box binding protein 1
XBP1-S  spliced XBP1
Zip1  zinc/iron regulated transporter-related protein 1

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