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Review

Alzheimer disease: Amyloidogenesis, the presenilins and animal models

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Abstract

Alzheimer's disease is the most prevalent form of dementia. Neuropathogenesis is proposed to be a result of the accumulation of amyloid beta peptides in the brain together with oxidative stress mechanisms and neuroinflammation. The presenilin proteins are central to the gamma-secretase cleavage of the amyloid prescursor protein (APP), releasing the amyloid beta peptide. Point mutations in the presenilin genes lead to cases of familial Alzheimer's disease by increasing APP cleavage resulting in excess amyloid beta formation. This review discusses the molecular mechanism of Alzheimer's disease with a focus on the presenilin genes. Alternative splicing of transcripts from these genes and how these may function in several disease states is discussed. There is an emphasis on the importance of animal models in elucidating the molecular mechanisms behind the development of Alzheimer's disease and how the zebrafish, *Danio rerio*, can be used as a model organism for analysis of presenilin function and Alzheimer's disease pathogenesis.
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1. Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia affecting more than 20 million people worldwide [1,2]. Furthermore, as a consequence of the worlds aging population, the prevalence of AD is expected to increase [1]. This highlights the importance of research investigating the mechanisms behind the development of the disease. Apart from the recognisable behavioural differences, it is difficult to positively diagnose someone with Alzheimer's during the early stages of the disease [1]. However the AD brain shows a consistent pathology amongst patients, with amyloid aggregates and neurofibrillary tangles evident in the AD brain. Intense research has identified genes involved in the development of these pathologies, namely the amyloid precursor protein and the presenilins. The increasing knowledge on AD pathogenesis has revealed the complex nature of this disease and highlights the need to elucidate the molecular mechanisms involved. This review discusses the current knowledge of the molecular mechanisms of Alzheimer Disease, focusing on amyloidogenesis, the role of the presenilin genes and the importance of animal models to further elucidate the mechanisms behind the development of the disease.

The most common form of AD in the population (approximately 90%) occurs sporadically and is late in onset, usually occurring after 65 years of age [1,2]. Familial Alzheimer's disease (FAD), only accounts for approximately 10% of cases and symptoms usually occur before the age of 65 [1]. The mode of inheritance of AD differs for each type. The majority of FAD and sporadic AD cases have a complex inheritance, while only 10% of FAD cases are inherited in an autosomal dominant pattern [3]. In affected individuals the disease causes a progressive and permanent decline in memory and cognitive abilities. The first cognitive area affected is episodic memory [4]. During disease progression, attention, executive functions, semantic memory, language and spatial orientation all begin to deteriorate [5]. However the molecular, cellular and pathological triggers for the onset of the cognitive deterioration are poorly understood.

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2. The pathogenesis and genetics of Alzheimer's disease

Alzheimer's disease was originally recognised by Alois Alzheimer in 1907 as a separate form of dementia. Since this original observation the two main histological features of amyloid plaques and neurofibrillary tangles (NFT) have been described in the AD brain [6]. These features are found to be present in the temporal neocortex and hippocampal regions of the AD brain [7]. The hippocampus resides in the cerebral cortex of the forebrain and is thought to be involved in memory storage. Amyloid plaques and NFTs result from an aberration in deposition of the amyloid beta peptide (Aβ peptide) and the hyperphosphorylated tau protein respectively and these depositions lead to neuronal loss and neurotoxicity in the AD affected brain [8]. Accumulation of Aβ peptides may be the key event in pathogenesis of AD. The exact mechanism by which Aβ peptide deposition induces neurotoxicity is unclear, but it appears the oxidative stress plays an important role. Oxidative stress is extensive in AD [9,10] and Aβ peptides stimulate oxidative stress by both direct and indirect mechanisms. Aβ peptides by themselves may act as enzymes [11], as they are capable of directly producing hydrogen peroxide and generating free radicals through metal ion reduction [12,13]. As well, Aβ peptide can bind to mitochondrial proteins resulting in the generation of free radicals [14]. Furthermore, Aβ peptides generate oxidative stress via neuroinflammation. Considerable evidence has supported that neuroinflammation is associated with AD pathology [15]. The death of neurons observed in AD is partly attributed to the activation of two major brain cell types, astrocytes and microglia that participate in the immune/inflammatory response to Aβ deposition. However, these cells can have both neuroprotective and neurodegenerative functions. For example, astrocytes cluster at sites of amyloid deposition and are thought to be involved in the clearance of plaques, evident through their ability to degrade amyloid beta deposits *in vitro* and *in situ* [16]. Both astrocytes and microglia release pro-inflammatory molecules upon activation, in particular, astrocytes can be activated by amyloid beta deposits to produce reactive oxygen species, as well as chemokines, cytokines, that lead to neuronal cell death [17]. Aβ peptides also induce the expression of nitric oxide synthase in microglia and the release of reactive nitric oxide which results in the loss of selected neuron populations [18]. Furthermore, Aβ peptides stimulate microglial cells to release a neurotoxin, quinolinic acid [19], which may also play a role in neurotoxicity.

2.1. The amyloid cascade hypothesis

The amyloid beta hypothesis predicts that an aberration in the proteolytic processing of the amyloid precursor protein (APP) leads to the increased production of Aβ peptides, this in turn leads to the accumulation of Aβ, a primary event in the pathogenesis of AD [1]. The accumulated Aβ eventually deposits into amyloid plaques present in the AD brain parenchyma. Together with the secondary events of microglial and astrocyte activation and NFT formation there is widespread neuronal dysfunction and selective neuronal loss. Currently, this hypothesis is being referred to as the Aβ cascade hypothesis, where increased production and possibly decreased clearance of Aβ peptides leads to an accumulation of these peptides and the subsequent pathogenesis observed in AD.

APP is a ubiquitously expressed type I integral membrane glycoprotein with various major isoforms. The largest of the APP alternate transcripts comprises an alternatively spliced exon at residue 289 which contains a serine protease inhibitor of the Kunitz type (KPI) [20]. APP resides on chromosome 21 and Down's syndrome cases (caused by an extra copy of 21) have been shown to develop early onset dementia. This observation, in conjunction with other studies lead to the discovery of the first reported partial amino acid sequence of Aβ in 1984 [21]. APP is expressed abundantly in a variety of tissues and processing of APP is a normal event in nearly all neural and non-neural cells throughout the body. Proteolytic processing of APP that releases the Aβ fragment is a result of cleavage events by secretase proteins. APP processing by the secretase proteins occurs in two distinct pathways, the non-amyloidogenic and amyloidogenic pathways. Soluble APP is cleaved by α-secretase and β-secretase to release the soluble α-APP and β-APP, respectively, into the extracellular matrix [22]. In the non-amyloidogenic pathway, γ-secretase cleavage of the remaining C-terminal fragment (CTF) releases the p3 fragment, while in the amyloidogenic pathway this cleavage releases the Aβ peptide (Fig. 2). The cleavage by γ-secretase is a heterogenous event that releases Aβ peptides of different sizes, with Aβ-40 and Aβ-42 being the most common forms. Aβ-40 and Aβ-42 are both toxic peptides, however the Aβ-42 isoform is insoluble and more capable of aggregating into amyloid plaques. Upon accumulation of Aβ-42 there is a resulting neuronal cytotoxicity that induces neuropathological events leading to neurodegeneration of the brain [23]. More specifically, Aβ-42 can aggregate into two different conformation states. There is the non-β sheet, non-fibrillar state and the β sheet fibrillar state which is cytotoxic and eventually deposits into plaques [24]. Aβ-42 exists initially as a monomer, during oligomerisation the monomers further oligomerise into larger forms such as large oligomers, protofibrils and fibrils. During oligomerisation there are conformational changes occurring in these elements that transform them into a β sheet fibrillar state [23]. The Aβ oligomeric intermediates (oligomers, protofibrils) and the mature fibrils are all neurotoxic, and it has been demonstrated that the oligomers and protofibrils are actually more neurotoxic than the mature fibrils or amyloid plaques [25].

2.2. Genes implicated in Alzheimer's disease

Three genes that have been implicated in AD pathogenesis are APP, presenilin 1 (PS1) or presenilin 2 (PS2), as mutations in these genes have been identified in cases of familial AD [26]. PS1 is central to the γ-secretase complex of proteins that cleave Notch and APP [27]. It is the presenilin-mediated cleavage of APP that results in the release of various lengths of the Aβ peptide. The pathological characteristics of FAD and the sporadic form of AD are proposed to be similar; therefore somatic changes in APP, PS1 or PS2 may potentially have a role

in sporadic cases of AD. Along with causative mutations in PS1, PS2 and APP, the apolipoprotein (APOE) gene has been identified as a major genetic risk factor for sporadic AD. E4 is an isoform of APOE and carriers of the E4 allele are at a higher risk of developing AD, with homozygotes usually developing AD earlier than heterozygotes [28]. However the presence of the APOE4 allele, has not been found to be necessary or sufficient for the development of the disease [28].

The gene encoding APP was the first to be identified has having an association with FAD. Currently there are 27 mutations in the APP gene that have been described in families with AD and these mutations are clustered around the Aβ sequence on APP [29]. Mutations in PS1, PS2 and APP account for approximately 50% of Familial AD cases and there is almost 100% penetrance of the mutated gene product that leads to AD development [29]. Worldwide studies on FAD cases have uncovered 157 and 10 pathogenic mutations in the PS1 and PS2 genes respectively (http://www.molgen.ua.ac.be/ADMutations/)[30]. The presenilin section of this review discusses presenilin associated FAD mutations in more depth.

In vitro and *in vivo* models of familial AD mutations have demonstrated an enhancement of APP proteolytic processing through aberrations of the γ-secretase complex [31,32]. These alterations generally increase the production of Aβ-42 or decrease the production of Aβ-40, which results in changes to the Aβ-40/Aβ-42 ratio, an important aspect of AD [33,34]. A shift in APP processing to release the longer Aβ-42 isoform leads to Aβ fibril formation [32,35]. These mutations usually have a familial inheritance pattern. For example, a mutation in APP identified in a Swedish family alters two amino acids that precede the Aβ amino terminus and results in enhanced β-secretase cleavage, causing an increase in Aβ production [36]. Aβ peptides with the Flemish mutation assemble into fibrils and protofibrils at a slower rate compared to wild type [37], while in the Dutch mutant, Aβ protofibrils and fibrils are formed at a much faster rate than wild type [38]. Theuns et al. [33] have to date reported the furthermost C-terminal APP K724N mutation. *In vitro* expression analysis of this mutation displayed an increase in Aβ-42 and decrease in Aβ-40 levels, which increased the Aβ-40/Aβ-42 ratio three-fold [33]. Even though these mutations are located on adjacent codons on APP, they produce different pathological characteristics in the patient population.

2.3. Linking oxidative stress with Alzheimer's disease

Oxidative stress is associated with a wide range of disease states including cancer, diabetes and neurodegenerative disorders such as AD. Oxidative stress results from a disturbance in the balance of antioxidants and reactive oxygen species (ROS) generated within the body, resulting in an excess of ROS. This leads to the destruction of both neuronal and vascular cells [11]. Oxidative stress disturbs the normal functioning of cells through cell membrane lipid destruction and cleavage of DNA [39]. Whether oxidative stress is involved at the onset of AD is still unclear. However it is thought to play a significant role during disease progression, particularly in cellular and tissue damage that occurs throughout AD. As noted above, oxidative stress is extensive in AD. It has also been suggested that there is a close correlation between oxidative stress and Aβ deposition, evident through mouse brain Aβ deposits co-localising with a variety of oxidative stress markers [40]. Aβ is proposed to be a metalloenzyme that is capable of generating hydrogen peroxide through its superoxide dismutase activity [11]. Hydrogen peroxide is capable of being further transformed to the reactive hydroxyl radical in the presence of transition metals, following this transformation lipid peroxidation reactions bring about oxidative damage, leading to oxidative toxicity in neurons [11]. It has been further suggested that oxidative stress may actually promote the amyloidogenic pathway [11], the resulting increase in Aβ can in turn generate more hydrogen peroxide leading to further oxidative damage in neurons and vascular cells. A cyclic pathway of excess Aβ promoting oxidative damage which promotes further Aβ production would result in heightened neurotoxicity and subsequently enhance the development of AD [11]. As discussed above, Aβ can produce oxidative damage by stimulating neuroinflammation, as well as generating ROS as a result of binding to mitochondrial proteins. Furthermore, intermediate amyloid aggregates can interact with cell membranes, where they form non-specific ion channels, with the resultant increases in intracellular calcium triggering the production of ROS [41]. Thus there is a strong link between Aβ, oxidative stress and AD (see Fig. 1).

2.4. Alternative initiating mechanisms for the amyloid cascade hypothesis

Apart from the identified FAD point mutations there is evidence that other neurochemical factors may initiate the deposition of Aβ in FAD or sporadic AD. The following are

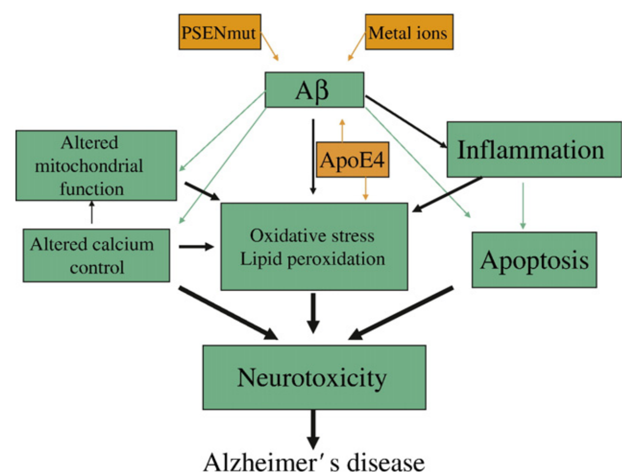

Fig. 1. Amyloid ß-protein (Aß) can accumulate through over production (e.g., presenillin mutation [PSENmut]) or reduced removal (e.g., APOE4). Oligomerization of Aß results in production of oxidative stress by itself (which can be augmented by heavy metals), of via induction of several other interacting mechanisms.

some of the current alternate mechanisms that possibly contribute to or initiate the development of neuropathological features of AD.

2.4.1. The role of vascular dysfunction and risk factors for AD pathogenesis

Cardiovascular disease and type 2 diabetes are risk factors for AD. Vascular risk factors such as hypertension and hypercholesterolemia are proposed to promote the production of Aβ [42,43], while being overweight or obese is also now associated with AD [44,45]. Vascular risk factors appear to be exacerbated by the inheritance of the E4 allele of the APOE gene, thus the presence of this allele increases the risk of developing both vascular dysfunction and AD [43]. A recent study by Balakrishnan et al. [46] investigated whether being overweight or obese contributes to AD development by modulating Aβ levels. In their study of 18 adults they found a correlation between the direct measures of obesity and plasma levels of Aβ-42 [46]. This is the first study to suggest that being overweight and obese is a risk factor for developing AD by increasing plasma levels of Aβ-42.

Recent findings have also suggested that neurovascular dysfunction is an important feature of chronic neurodegeneration in AD [47]. The neurovascular hypothesis as suggested by Zlokovic et al. [47] proposes that there is faulty clearance of Aβ across the blood brain barrier (BBB). In one instance the faulty clearance, possibly due to aberrant angiogenesis, results in increased Aβ levels. Furthermore, another pathway could involve increased Aβ concentration in conjunction with atherosclerosis. Together, these mechanisms are proposed to cause premature aging of the cerebrovascular system [47]. Both of these cascades could initiate neurovascular uncoupling, vessel regression, brain hypoperfusion and neurovascular inflammation [47]. Flow on effects include compromise of the BBB, an ionic imbalance and metabolic poisoning in the brain interstitial fluid, finally leading to synaptic dysfunction and neuronal injury and loss, evident pathologies in AD [47].

2.4.2. Redox active metals are key mediating factors in the pathophysiology of AD

It is likely that there is a pathological interaction of Aβ with redox active metals such as zinc, copper and iron, due to these metals having a high concentration in the areas of the brain that are affected by AD, namely the cortex, hippocampus and cortical vasculature [48,49,50]. Redox active metals may play a role in oxidative stress as they influence the production of hydrogen peroxide [24] (see Fig. 1). The binding of trace levels of these metals to Aβ, promotes the catalytic production of hydrogen peroxide from oxygen through metal reduction [13]. The effect of these metals on the oxidative mechanisms of Aβ cytotoxicity is complex and not yet fully elucidated, however the effect of zinc on AD pathology may be explained by 'The Zinc Paradox' [24]. During normal physiology, zinc, copper and to a lesser extent iron interact with APP and Aβ, probably existing in a delicate balance. Perturbations in this balance may result from abnormal Aβ metabolism and synaptic zinc flooding, resulting in a change in zinc and copper metabolism leading to the formation of fibrillar Aβ. Consequently, this fibril formation triggers an inflammatory response that lowers pH and leads to a disruption of zinc homeostasis [24]. Zinc is released into the cellular environment and this triggers events that lead to oxidative stress-induced cell death [51]. Copper ions are then free to compete for zinc's binding site on Aβ and these ions can then catalyse hydrogen peroxide production by Aβ. Zinc is essential in human nutrition; however the role of cerebral zinc in AD pathology requires further elucidation. Since it has been established that zinc is elevated in cored amyloid plaques [52] and zinc flooding can lead to increased Aβ fibril formation and oxidative stress, zinc may, paradoxically, be a pathological factor for AD.

3. The presenilin proteins

In humans the *PS1* gene resides on chromosome 14 [53] and the *PS2* gene resides on chromosome 1 [54,55]. Presenilin proteins are evolutionarily conserved amongst species. Presenilins are transcribed in both the central nervous system and in non-neuronal organs. Presenilin functions within the γ-secretase complex, with the mature complex predominantly existing at the cell surface regulating the cleavage of a number of single transmembrane domain proteins [56]. Furthermore presenilin (as a part of the γ-secretase complex) is also found to reside in the membranes of the endoplasmic reticulum and Golgi apparatus [57]. *In vitro* generated point mutations in the *PS1* gene leads to an increased production of Aβ peptides [58,59,60] and there is a reduction in γ-secretase cleavage of APP in neuronal cultures derived from *PS1* deficient mice embryos [61]. These findings and more led to the conclusion that mutations in *PS1* have a gain-of-function phenotype and that PS1 is a target for anti-amyloidogenic therapy in AD. It now is well substantiated that presenilins functions in the γ-secretase cleavage of APP, however, the complete function of PS1 and PS2 has not yet been determined. PS1 and PS2 are highly homologous and share 62% amino acid identity. Upon deletion of PS1 and PS2 in the forebrain of mice, there is a severe phenotype that resembles a Notch gene knockout [62]. There are also evident biochemical alterations of neuronal atrophy, astrogliosis, caspase-3-mediated apoptosis and tau hyperphosphorylation [63]. Together these alterations demonstrate that the presenilins are essential for the ongoing maintenance of cortical structures and function in the brain [63]. Presenilins are also involved in several cell-signalling pathways with an essential function during development [64] and they regulate β-catenin stability and trafficking of membrane proteins [65,66]. Presenilin has also been implicated in calcium signalling. Tu et al. [67] recently demonstrated the presenilin holoprotein accounting for ~80% of in Ca^{2+} leakage from the ER. Interestingly it appears to be independent of its γ-secretase activity [67].

Presenilins have also been implicated in different apoptotic pathways. A partial cDNA clone encoding a portion of PS2 CTF rescued a T-cell receptor and FAS-induced apoptosis [68]. Furthermore, PS is implicated in mitochondria-dependent apoptotic cell death, through its interactions with the anti-

apoptotic proteins Bcl-2 [69] and Bcl-X_L [69,70]. Even though presenilins' involvement in apoptotic processes has been demonstrated, the actual mechanism of their apoptotic function is yet to be elucidated (Fig. 2).

The SEL-12 protein, a presenilin homologue, is expressed throughout the nematode *Caenorhabditis elegans* (*C. elegans*). Due to the almost identical function of SEL-12 and human PS1, the study of SEL-12: LacZ fusion proteins has predicted the topology of the PS1 protein to encompass eight transmembrane (TM) domains, with a hydrophilic loop between the sixth and seventh domain [71]. Other topological studies using a similar experimental design that probes for membrane topology by using the glycosylation of PS1 hybrid proteins, have suggested models of the presenilin protein ranging between 6 and 9 TM domains [72–75]. The conserved aspartate residues Asp 257 in TM6 and Asp385 in TM7, appear to contain the active site of PS1 that contributes to γ-secretase activity and the region between these two residues is critical for PS1 endoproteolysis. It has been demonstrated that mutations in either of the two aspartate residues prevent endoproteolysis of PS1 in the cytoplasmic loop, substantially reduce the production of Aβ and decrease Aβ secretion by 60% [76], while mutating an equivalent aspartate in PS2 results in undetectable levels of Aβ [77]. The major endoproteolysis site of the PS1 holoprotein has been identified as residue 298 located in the large hydrophilic loop [78].

Presenilin is translated as a full-length holoprotein which is predominantly endoproteolytically cleaved by presinilinase into the 27-kDa N-terminal (NTF) and 17-kDa CTF [79]. The site of endoproteolysis has been shown to be in the exon 9 hydrophilic loop region, since a PS1 variant lacking exon 9 did not undergo endoproteolysis [79]. Endoproteolysis appears to be the critical event that brings about PS1 stability and biological activity as antibody staining of specific sections of presenilin has indicated that there is very little full-length PS1 or PS2 protein detected in brain tissue [78]. Ratovitski et al. [80] demonstrated that presenilin NTF and CTF are significantly more stable then the full-length polypeptide in mouse cell lines expressing human presenilin. Thus, presenilin is mainly present as N- and C-terminal endoproteolytic fragments. These fragments dimerise to form the active, functional presenilin heterodimer [78]. As a consequence of the heterodimer formation the fragments are protected from the relatively rapid degradation that is evident for the holoprotein [81]. PS1 is able to form both low (100–180 kDa) and high (>250 kDa) molecular weight complexes that can contain the full length or the heterodimeric CTF and NTF fragments [81,82]. Furthermore the formation of the higher molecular weight complex appears to be required for presenilin mediated cleavage of APP and the signal receptor Notch (described in detail later). Hebert et al. [83] have proposed a hypothetical model of PS1 maturation that is in accordance with a model proposed by Haas and Steiner et al. [84]. In this model, PS1 homodimerises before the full-length protein is endoproteolytically cleaved by presinilinase. A tetramer of hetero and homodimers would then be required for the assembly and stability of the high molecular weight complex needed for γ-secretase activity [83]. Through yeast two hybrid and split-ubiquitin assays, Cervantes et al. [85] have also observed NTF: NTF and CTF: CTF dimers. Subsequently, a tetramer model of the presenilin functional complex was proposed and further analysis of the effect of FAD mutations on NTF: NTF homodimerisation displayed these mutations affecting the NTF: NTF interaction. This result implies that FAD mutations influence the assembly or possibly the stability of the functional presenilin complex.

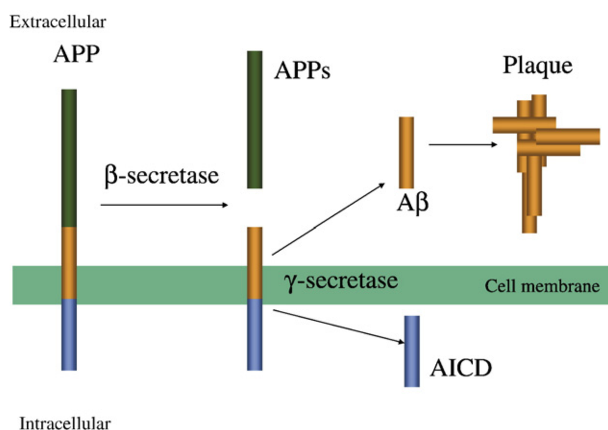

Fig. 2. Processing of APP in the amyloidogenic pathway. Cleavage of APP by secretase enzymes releases the amyloid beta peptide, excess peptides eventually deposit into amyloid plaques.

3.1. Presenilin is integral to the γ-secretase complex

γ-Secretase is a transmembrane aspartyl protease that exists as a mature complex within the plasma membrane, where it interacts with and cleaves single transmembrane receptors such as Notch and APP [56]. Prior to cleavage these substrates undergo a shedding of a large ectodomain leaving a membrane-embedded CTF. The CTF is subject to intramembrane cleavage by the γ-secretase complex, the proteolytic cleavage releases an intracellular domain that is able to modulate transcription [86].

Findings by Kimberly et al. [87] suggest that four membrane proteins comprise the γ-secretase complex and these enzymes co-assemble to form the active complex in mammalian cells. Currently it is thought that the higher molecular weight complex that PS1 forms contains the proteins nicastrin (NCT), anterior pharynx defective (APH-1) and presenilin enhancer 2 (PEN2). Through multistep affinity purification of γ-secretase, the PS, NCT, APH-1 and PEN-2 proteins were shown to co-purify as a complex [88]. Overexpression studies of APH1a, APH1b, PEN2 and NCT DNA in HEK293 cells, resulted in an increase in the production of Aβ-40 and Aβ-42, suggesting that these proteins are limiting for γ-secretase activity. Overexpression of APH-1 in S2 cells of the fruit fly *Drosophila melanogaster* results in a marked increase in the PS1 holoprotein that is further enhanced by overexpression of NCT [89]. RNAi depletion of PEN2 in S2 cells, mammalian neuronal cells and mouse neuroblastoma cells results in an accumulation of the full length PS protein, thus preventing endoproteolysis [89]. Furthermore, RNAi-mediated inactivation combinations in S2

cells of PEN2 and NCT or PEN2 and APH-1 totally abolished this accumulation [89]. Interestingly, when NCT is deleted in *C. elegans* or *Drosophila*, the resulting phenotypes are similar to the loss of PS1 or Notch [90,91]. Cleavage of APP and Notch at the γ-secretase cleavage site is also eliminated, together with a loss of PS1 endoproteolysis and maturation [90]. This is also evident in NCT null mouse embryos. These embryos have severe developmental defects and die by embryonic day 10.5 [92]. Furthermore, embryonic fibroblast cells isolated from these NCT null mice have decreased levels of APH-1, PEN-2 and PS1 fragments together with a significant accumulation of full-length PS1 [93]. Collectively, these results implicate APH-1 as acting in the stabilisation of the PS holoprotein in the complex, while PEN-2 and NCT assist the endoproteolysis of PS1, thus providing γ-secretase activity. Recently, Chen et al. [94] discovered a new member of the γ-secretase complex, a type 1 transmembrane protein named TMP21 that is a member of the p24 cargo-family. It was isolated in a high molecular weight presenilin complex and was shown to interact with all of the complex components. They demonstrated that overexpression of TMP21 had no effect on the levels of the other complex components or Aβ production, however suppression by small interfering RNAs increased the production of Aβ40 and Aβ42. Thus, through its interaction within the γ-secretase complex TMP21 appears to modulate Aβ production [94]. PS1 has homology to part of an active site of a known bacterial aspartyl protease [95].

Notch proteins are a group of large cell-surface receptors that are required for cell-fate decisions during development. Following binding to its ligand on the cell surface, Notch-1 undergoes γ-secretase cleavage at site 3 (S3) within its intracellular domain to release the Notch intracellular domain (NICD) fragment [96]. NICD then translocates to the nucleus and interacts with DNA-binding proteins to regulate transcription. Mutations in the SEL-12 protein of *C. elegans* result in an egg laying defect, indicating a loss of Notch function. Expression of human PS1 or PS2 in these mutants rescues the egg laying defect. However, in the presence of FAD point mutations there is an observed reduction in rescue [65]. Knocking out PS in *Drosophila* results in phenotypes identical to Notch null mutants [97]. Therefore, PS is required for Notch function and presenilin's function in notch signalling is conserved between humans, *C. elegans* [98] and *Drosophila*.

A diverse range of additional cell surface proteins have been identified as γ-secretase and presenilin substrates. There is little in common between these substrates except that they all posses single transmembrane domains. Deleted in colon cancer (DCC), ErbB4, Delta and p75 neurotrophin receptor (p75NTR) are γ-secretase substrates known to influence neuronal structure and function and are important for development of the nervous system. However the role of cleavage of these proteins by γ-secretase is unknown [99]. Even though it can be postulated that perturbations in the γ-secretase mediated cleavage of these proteins may affect neuronal function, currently an association of these molecules with neurodegeneration or AD development has not be established.

3.2. Presenilin mutations and alternative splicing in Alzheimer's disease

The majority of missense mutations identified in FAD occur at highly conserved sites [100] and are spread throughout the gene. The mutations identified in PS1 are spread through TM domains 1–6 and also in the N and C-terminus, while in PS2 the FAD mutations are present in TM domains 2 and 5 and a mutation identified in a sporadic case of AD is found at the N-terminus [29]. Interestingly, approximately 21% of PS1 AD associated mutations and 4 out of 10 reported PS2 mutations are found in exon 5 [64]. Therefore this region may hold some importance in regard to structure and function of presenilin within the lipid bilayer as exon 5 resides in transmembrane domain 2 of PS1 and PS2 [64]. Presenilin mutations also disturb protein interactions in the γ-secretase complex through conformation changes that affect APP processing [29] and alter the Aβ40/42 ratio either through in an increase in Aβ-42 or a decrease in Aβ-40 [33,34,60]. This is evident through a consistent overproduction of Aβ-42 peptides in brain tissue of FAD patients [29] and transgenic mice harbouring a human PS1 mutation [3]. Furthermore, cultured cells transfected with mutant PS1 or PS2 have a 1.5 to 5 fold increase in the intracellular concentration of Aβ 1–42 compared to cells with wild type PS1 or PS2 [29].

The alternative splicing of exons within a gene, results in the production of different protein isoforms. The isoforms may share or differ in function from the original protein [101]. Splice variants have been identified in PS1 and PS2. In human brain tissue of sporadic AD patients a splicing variant of PS2 (PS2V) has been detected. This variant is preferentially expressed and lacks exon 5 [101]. Due to the exclusion of exon 5 a frameshift occurs in exon 6, resulting in a PS2V containing residues Met1 to Leu119 which is encoded by the amino terminal and a further five additional residues at its carboxyl terminus [102]. In the study by Sato et al. [101] 83% of the variant positive cases had sporadic AD and in neuroblastoma cells the spliced variant was detected under hypoxic conditions. Upon antioxidant treatment, there was an inhibition of the hypoxia induced spliced variant [101]. Interestingly a study by Matsuzaki et al. [103] showed that the PS2V induced by hypoxia can be accelerated by chronic exposure to aluminium. The resulting cell death from this exposure is due to the hypoxic conditions and can be partly attributed to oxidative stress. Considering that aluminium is not a necessary metal for cell function, this result may indicate that chronic aluminium exposure is an environmental risk factor for the development of AD [103].

Kwok et al. [104] discovered an early-onset FAD case that had a novel missense mutation (L271V) present in PS1. Exon trapping analysis confirmed that this mutation results in altered exon 8 splicing and a variant neuropathology in the pedigree. Neuropathological analysis demonstrated the presence of large non-cored plaques, which indicate diffuse plaques rather then the typical neuritic amyloid plaques. Western blots of cell lysates immunoprecipitated with anti-tau or antiGSK-3β indicated that wildtype but not exon 8-deleted PS1 is able to interact directly with Tau and GSK-3β. This supports the idea

that the exon 8 region of PS1 interacts with these proteins. Since GSK-3β phosphorylates tau and initiates neuritic dystrophy, PS1 exon 8 appears to be necessary for neuritic plaque formation. Biochemical analysis indicated that this mutation increased the production of the Aβ-42 peptide [104]. A novel variant of PS1 in an AD Finnish pedigree has also presented with diffuse plaques. Through molecular analysis it was demonstrated that this variant had a deletion of exon 9 [105]. Therefore, it appears that the loss of either exon 8 or 9 results in AD with diffuse plaques lacking the dense neuritic core of Aβ, implying that this region of presenilin has some role in neuritic plaque development. The presence of diffuse rather then neuritic plaques in these cases provides further evidence that the pathogenic events of AD occur before the aggregation of Aβ into neuritic plaques.

3.3. Aberrant presenilins are implicated in other disease states

Aberrant splicing events in the PS1 gene have been observed in other neurological disorders. The presence of PS1 gene products that have deletions in the exons 4–8 region have been found in the brain of sporadic cases of frontotemporal dementia (FTD) [106]. As previously mentioned this disorder has NFTs present in the brain with no detection of amyloid plaques. Furthermore, the increased expression of the PS2V isoform detected in the brains of sporadic AD patients, has also been detected in the cortex at significantly elevated levels in some bipolar and schizophrenia cases [64] A novel mutation in PS1 (Gly183Val) was identified in a patient with a Pick type tauopathy, with the absence of Aβ plaques [107]. This mutation affects the splice signal of the sixth exon intron junction, which may result in aberrant splicing. Preliminary analysis of alternatively spliced products revealed a premature stop codon downstream of exon 5 [107]. These aberrantly spliced presenilins are toxic to normal presenilin function possibly through a loss of function or dominant negative affect. These findings implicate aberrant splicing of the presenilins in neurodegeneration together with its already documented role in development.

A casual link between neurodegenerative and cerebrovascular diseases has been consistently suggested when analysing these types of disorders, with vascular risk factors being identified has possible risk factors for AD pathogenesis [108]. Recently Rovelet-Lecrux et al. [109] demonstrated duplication of the APP locus in five families. These individuals develop early-onset AD with cerebral amyloid angiopathy (CAA). CAA is a disease where Aβ peptides deposit in the walls of small blood vessels in the brain leading to stroke, haemorrhages and dementia. Individuals with an APP duplication either present with dementia or intracerebral haemorrhages or a mixture of both phenotypes [109], thus suggesting a link between dementia (AD) and the vasculature of the brain. Missense mutations in the Notch 3 gene have been attributed to the vascular disease, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) [110]. The common symptoms of this disorder are migraines, ischemic strokes, mood disorders and progressive cognitive impairment that results in dementia [111]. Along with dementia progression, a further link to neurodegenerative disorders is evident though recent research identifying the basis of CADASIL as being a degenerative disorder of brain vessels [108]. Interestingly, the intramembrane region of Notch 3 possibly undergoes presenilin mediated γ-secretase cleavage, similar to the cleavage events of APP in AD pathogenesis. The role of Notch in vascular integrity and the role of presenilin in Notch signalling suggest the existence of molecular commonalities between these two diseases.

3.4. Presenilin regulation of the Wnt/β-catenin signalling pathway

The armadillo homologue, β-catenin is an effector molecule in the Wnt/Wingless signalling pathway. It is a transcription factor bound at the cell membrane, where it is involved in the maintenance of the link between the cadherins and the actin cytoskeleton [112]. β-catenin is also located in the cytoplasm in a complex with GSK3-β. Phosphorylation by GSK3-β promotes the ubiquitination of β-catenin, which in turn leads to the rapid degradation of β-catenin by the proteosome [113]. However GSK3-β can be inhibited by signalling through the soluble ligand, Wnt. Inhibition by Wnt results in the accumulation of unphosphorylated β-catenin in the cytoplasm, which eventually translocates to the nucleus. In the nucleus β-catenin binds to the LEF/TCF family of transcriptional regulators that activates target genes of Wnt.

Together with its role in intramembrane proteolysis of Notch and APP [27], PS1 also regulates protein degradation of β-catenin in the Wnt signalling pathway [114,115]. Co-immunoprecipitation experiments have demonstrated that β-catenin binds to the loop domain of PS1 coded by exons 8, 9 and 10 [116]. The functional relevance of this binding has been contradictory where PS1 FAD mutations have either increased β-catenin degradation [117] or interfered with the turnover of β-catenin leading to its accumulation [118]. Using PS1 knockout fibroblasts, Soriano et al. [115] convincingly demonstrated β-catenin accumulating in the cytoplasm with activation of LEF/TCF gene transcription. This indicates that PS1 negatively regulates Wnt/β-catenin pathway [115]. This is further supported by the evident accumulation of armadillo (*Drosophila* homologue of β-catenin) in presenilin-deficient *Drosophila* embryos [114]. It has been speculated that PS1 is needed for the phosphorylation of β-catenin, however Soriano et al. [115] demonstrated an accumulation of phosphorylated β-catenin in the PS1 deficient cells. Furthermore in the presence of a proteosome inhibitor there are lowered levels of ubiquitinated β-catenin observed in PS1 deficient cells compared to wild type cells, which suggested that PS1 is possibly required for the ubiquitination of β-catenin [115]. How PS1 affects the Wnt/β-catenin pathway and furthermore how Aβ production is affected by the interaction between PS1 and β-catenin still remains to be elucidated, though it does appear that this interaction functions in multiple signals that lead to cell fate decisions [35]. The loss of Wnt signalling does appear to play a role in AD [39], evident through the neurotoxicity of Aβ aggregation in hippocampal cells being linked to an increase in

the level of GSK-3β and a subsequent loss of β-catenin. As GSK-3β mediates the phosphorylation of tau, the increase in GSK-3β due to Aβ aggregation may contribute to the formation of NFTs. Overexpression of dishevelled, a downstream transducer of Wnt signalling, inhibits GSK-3β mediated phosphorylation of tau, demonstrating a possible role in increasing neuronal protection during neurodegenerative disorders together with its role in regulating notch signalling [119].

4. Animal models of Alzheimer's disease pathogenesis

Reliable animal models are required to facilitate the understanding of neurodegenerative pathways in AD. Models should allow for the testing of compounds at various points of the pathogenetic cascade in order to search for disease modifying drugs. Furthermore, they remain an invaluable tool for identifying molecular, cellular and pathological changes that trigger the onset of cognitive decline in AD. Specifically, the *presenilin* gene is highly conserved during evolution, with orthologues in mouse, *C. elegans* and *Drosophila* [100]. *Danio rerio*, more commonly known as zebrafish, has two orthologues, zebrafish *psen1* [120] and zebrafish *psen2* [121]. Transcripts from these genes have been demonstrated in zebrafish embryos, suggesting their requirement for development [122].

Drosophila and C. elegans models have been explored to investigate aspects of AD neuropathology. Recently Crowther and colleagues [123] developed a *Drosophila* model, in which human Artic mutant Aβ production is driven in the CNS and retina. This expression results in intracellular Aβ accumulation and non-amyloid aggregates that resemble diffuse plaques. These histological changes are associated with progressive locomotor deficits and premature death, indicating that the accumulation and aggregation of Aβ is sufficient to cause aspects of Aβ pathogenesis [123]. Moreover the fly phenotype is rescued by congo red, a dye that had been previously shown to reduce Aβ aggregation *in vitro* [123]. A study by Finelli et al. [124] examined the overexpression of Aβ-42 in *Drosophila* nervous system tissues. Overexpressing Aβ-42 in the eye resulted in a rough eye phenotype that worsened with age. There was also evidence of insoluble aggregates forming in the photoreceptors. Flies overexpressing Aβ-42 in the brain had a reduced life span in comparison to control flies [124].

C. elegans possesses three orthologues of human presenilin, sel-12 [98], hop-1 [125] and spe-4 [126,127]. Complete presenilin function is evident in the SEL-12 protein as human PS1 and PS2 substitute for SEL-12 activity in *C. elegans*. Also, as previously mentioned, the structure of the SEL-12 protein was used to determine the transmembrane domains of human PS1. In 1995, Link et al. [128] generated a *C. elegans* model of Aβ molecular pathology by genetically engineering animals to express human Aβ specifically in muscle cells. These nematodes produce muscle specific Aβ deposits that are immunoreactive with monoclonal and polyclonal anti-Aβ antibodies. The transgenic nematode larvae present with progressive muscle paralysis and vacuoles, phenotypes that can be attributed to toxicity in muscle cells [128].

4.1. Mouse models of AD neuropathology

There have been numerous transgenic mice developed that model phenotypes observed in AD. Oddo et al. [129] developed a triple-transgenic model in which cDNA from human APP Swedish mutation and human P301L Tau mutation were co-microinjected into embryos from homozygous mice with a human PS1 AD knock-in mutation [129]. This method generated triple transgenic mice with the same genetic background. Further studies showed that these mice present with amyloid plaques and NFTs in AD-relevant regions of the brain. The mice also develop dysfunctions in synaptic plasticity [130] in conjunction with the early intraneuronal accumulation of Aβ. More recently, this group has demonstrated that these mice present with an impairment of long-term memory retention at 4 months of age, indicating that intraneuronal Aβ may be involved in the onset of cognitive deterioration. Feng et al. [63] in reported studies on conditional deletion of PS1 and PS2 in the adult mouse. The loss of presenilin activity in adult mice resulted in forebrain degeneration, evident through cortical shrinkage, together with enlargement of the lateral and third ventricles. These are hallmark features in the brains of AD patients with advanced disease [63]. They also uncovered a series of cellular aberrations in these mice such as neuronal atrophy, astrogliosis, apoptosis and tau hyperphosphorylation. These results implicate the presenilins in the maintenance of cortical structures and functions [63].

In addition to these recent mouse models there are approximately 20 other strains of transgenic mice based on mutations in APP. However, the majority of these, particularly the Tg2576 mouse model (harbours the Swedish APP mutation), are missing important aspects of AD pathology, namely the neurofibrillary tangles [131]. Furthermore these transgenic mice present with memory loss but are lacking the evident neurodegenerative features of AD, thus, they resemble the latent phase of AD [131]. In comparison tau expressing transgenic mice develop neurofibrillary tangles, have evident neuronal loss and also present with other aspects of AD associated neurodegeneration [131]. In one particular line of tau transgenic mice containing the R406W tau mutation, aged transgenic mice present with memory impairment, without any obvious sensorimotor deficits [132]. Evidently there is a need to develop a model that mimics all aspects of the pathogenic cascade, from the triggering mechanisms of AD, to the latent phase of the disease.

4.2. The use of zebrafish to model Alzheimer's disease pathology

The zebrafish is an effective and simple model organism for studies of development and disease processes in the nervous system [133]. Developmental features of early embryonic patterning, early development of the nervous system and particular cell fates have been characterised in the zebrafish. The zebrafish as a vertebrate is more closely related to humans than invertebrate models such as yeast, worms or flies. The zebrafish is an advantageous model for genetic studies as it is

genetically malleable by injection of morpholino antisense oligonucleotides (MO), mRNA or transgenes. With these technologies it is possible to make both subtle and drastic changes in gene expression and observe the affects in the developing transparent embryo. MOs can be designed to block translation of a particular gene or to block the splicing of particular exons into transcripts. Overexpression studies can also be performed by injecting sense mRNA of a gene. For both MO and mRNA injection, the phenotypic affects on embryonic development usually only persist during embryogenesis (i.e. 2 days). Transgenic zebrafish can also be developed by using efficient vectors such as the sleeping beauty transposase system to insert genes under the control of tissue specific promoters. Furthermore, conditionally expressed transgenics can be generated using heatshock promoters and the Cre/loxP and GAL4-UAS systems for the analysis of gene function in specific tissues at specific times [134].

Zebrafish embryos are a valuable system in which to study presenilin function. Orthologues of *PS1* and *PS2* have been identified in zebrafish (*psen1* and *psen2*). Sequence alignment of zebrafish and human presenilin protein sequences displays these primary structures as being highly conserved, though there are highly variable regions at the N-terminus and the C-terminal half of the cytoplasmic loop domains. Transcripts from zebrafish *psen1* are ubiquitously expressed from fertilisation in the zebrafish. This implies that zebrafish *psen1* has an essential function throughout embryonic development [120]. Similar to full-length human *PSEN1*, zebrafish *psen1* cDNA stably transfected in HEK 293 cells, is rapidly turned over with very little being turned into stable CTFs [120]. However, *in vivo* the CTF concentration of zebrafish psen1 steadily increases during embryogenesis to 24 h post fertilisation (hpf), suggesting developmental regulation [122]. Zebrafish *psen2* mRNA is present from fertilisation but protein expression has only been detected from the onset of gastrulation (6 hpf).

Pen-2 is a critical member of the γ-secretase complex. It is thought to facilitate the endoproteolysis of PS1 [135], thus conferring γ-secretase activity. Both zebrafish Pen-2 and zebrafish Aph-1 (another member of the γ-secretase complex) are present from fertilisation similar to psen1. At 12 hpf both genes are ubiquitously expressed in the embryo and by 24 hpf they are expressed predominantly in the brain [136]. Injection of MO blocking Pen-2 translation led to a reduction in islet-1 positive neurons along the spinal cord, a reduction in Notch signalling, an impairment of somitogenesis and induced apoptosis throughout the whole fish [136]. The loss of islet-1 positive neurons in the spinal cord and the increase in apoptosis could be rescued by a concurrent knockdown of p53, implying that loss of Pen-2 induces a p53-dependent apoptotic pathway [136]. The p53-dependant apoptotic pathway contributes to neuronal loss, therefore Pen-2 may have a protective function against apoptosis and promote neuronal survival *in vivo*.

By modulating presenilin expression, it is possible to elucidate its function and regulation in zebrafish. DAPT is a known γ-secretase inhibitor that reduces Aβ peptide levels in the brain [137]. DAPT treatment impairs Notch processing through an interference of presenilin function [138] resulting in severe side affects of γ-secretase inhibitors on Notch dependent cell fate decisions [138]. Treatment of embryos with DAPT affects somitogenesis and neurogenesis by 24 hpf [138]. This phenotype is indistinguishable from Notch signalling deficiencies and can be rescued by injection of mRNA encoding the NICD fragment of zebrafish Notch1. Injection of MOs that block *psen1* transcript translation, leads to disruption of somite formation consistent with partial loss of Notch signalling in the presomitic mesoderm [122]. There is a disruption of myoD expression, a phenotype that is similar in mice lacking PS1 activity. Such mice have a milder phenotype than Notch1 null mutant mice and this has been attributed to the redundant activity of mouse PS2 [139]. However, PS2 knockout alone does not produce a notch phenotype [139]. The possible redundancy between mouse PS1 and PS2 in notch signalling is not reflected in the activity of zebrafish psen1 and psen2. Overexpression of zebrafish *psen2* by microinjection of *psen2* mRNA does not appear to regulate transcription of *psen1* in early zebrafish embryos (12 hpf) [122]. In contrast to observations in mouse cell culture [140], overexpression of *PS2* also does not alter the level of endogenous PS1 holoprotein [122]. Indeed, similar for psen1, repression of psen2 function in zebrafish embryos provides notch loss of function phenotypes [141]. These studies indicate that zebrafish embryos are a valid and valuable system for the study of presenilin function and regulation.

Two homologues of APP, *appa* and *appb* have been identified in zebrafish. Both genes have approximately 70% amino acid identity to human APP-695, with 80% identity in the Aβ-42 region and 95% identity within the transmembrane domain [142]. Widespread expression of both genes is first detected at 8 hpf but at 10 hpf the distribution of expression of two genes diverges. By 24 hpf both genes have overlapping but distinct patterns of expression. Overall the expression pattern of these two genes is similar to that observed for the mouse APP-695 isoform [143]. This indicates that APP gene function is conserved during vertebrate evolution.

Zebrafish are useful for studies of the effects of environmental hazards on vertebrate cells. Similar to mammals, the zebrafish have defence mechanisms against toxic chemicals that includes the generation of oxidative stress [144]. Carvan et al. [144] treated zebrafish and human cell lines with a variety of chemicals known to induce transcription of certain protective genes in cultured mammalian cells. They observed activation of transcription of these genes in a similar dose dependent manner in the zebrafish and human cells, indicating this process to be well conserved between mammals and zebrafish [144].

Alzheimer's disease is clearly a complex, heterogenous disorder evident through extensive research spanning from oxidative stress mechanisms to vascular dysfunction. Intense research has identified genes and environmental factors that heighten the risk of developing AD. The pathogenesis of AD may occur through several pathways, with the common characteristics of Aβ deposition and to a lesser extent aberrant presenilins, which have also been demonstrated in other neurodegenerative disorders. Easily manipulable vertebrate

models, now including the zebrafish, are proving to be increasingly important in elucidating these pathways.

References

[1] K. Blennow, M.J. de Leon, H. Zetterberg, Alzheimer's disease, Lancet 368 (2006) 387–403.

[2] D. Pratico, N. Delanty, Oxidative injury in diseases of the central nervous system: focus on Alzheimer's disease, Am. J. Med. 109 (2000) 577–585.

[3] D.J. Selkoe, M.B. Podlisny, Deciphering the genetic basis of Alzheimer's disease, Annu. Rev. Genomics Hum. Genet. 3 (2002) 67–99.

[4] S. Artero, M.C. Tierney, J. Touchon, K. Ritchie, Prediction of transition from cognitive impairment to senile dementia: a prospective, longitudinal study, Acta Psychiatr. Scand. 107 (2003) 390–393.

[5] M.A. Lambon Ralph, K. Patterson, N. Graham, K. Dawson, J.R. Hodges, Homogeneity and heterogeneity in mild cognitive impairment and Alzheimer's disease: a cross-sectional and longitudinal study of 55 cases, Brain 126 (2003) 2350–2362.

[6] D.J. Selkoe, The molecular pathology of Alzheimer's disease, Neuron 6 (1991) 487–498.

[7] K. Taguchi, H.D. Yamagata, W. Zhong, K. Kamino, H. Akatsu, R. Hata, T. Yamamoto, K. Kosaka, M. Takeda, I. Kondo, T. Miki, Identification of hippocampus-related candidate genes for Alzheimer's disease, Ann. Neurol. 57 (2005) 585–588.

[8] C.W. Cotman, J.H. Su, Mechanisms of neuronal death in Alzheimer's disease, Brain Pathol. 6 (1996) 493–506.

[9] M. Aslan, T. Ozben, Reactive oxygen and nitrogen species in Alzheimer's disease, Curr. Alzheimers Res. 1 (2004) 111–119.

[10] D.A. Butterfield, S. Griffin, G. Munch, G.M. Pasinetti, Amyloid beta-peptide and amyloid pathology are central to the oxidative stress and inflammatory cascades under which Alzheimer's disease brain exists, J. Alzheimers Dis. 4 (2002) 193–201.

[11] C. Opazo, X. Huang, R.A. Cherny, R.D. Moir, A.E. Roher, A.R. White, R. Cappai, C.L. Masters, R.E. Tanzi, N.C. Inestrosa, A.I. Bush, Metalloenzyme-like activity of Alzheimer's disease beta-amyloid. Cu-dependent catalytic conversion of dopamine, cholesterol, and biological reducing agents to neurotoxic H(2)O(2), J. Biol. Chem. 277 (2002) 40302–40308.

[12] X. Huang, M.P. Cuajungco, C.S. Atwood, M.A. Hartshorn, J.D. Tyndall, G. R. Hanson, K.C. Stokes, M. Leopold, G. Multhaup, L.E. Goldstein, R.C. Scarpa, A.J. Saunders, J. Lim, R.D. Moir, C. Glabe, E.F. Bowden, C.L. Masters, D.P. Fairlie, R.E. Tanzi, A.I. Bush, Cu(II) potentiation of alzheimer abeta neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction, J. Biol. Chem. 274 (1999) 37111–37116.

[13] X. Huang, C.S. Atwood, M.A. Hartshorn, G. Multhaup, L.E. Goldstein, R.C. Scarpa, M.P. Cuajungco, D.N. Gray, J. Lim, R.D. Moir, R.E. Tanzi, A.I. Bush, The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction, Biochemistry 38 (1999) 7609–7616.

[14] J.W. Lustbader, M. Cirilli, C. Lin, H.W. Xu, K. Takuma, N. Wang, C. Caspersen, X. Chen, S. Pollak, M. Chaney, F. Trinchese, S. Liu, F. Gunn-Moore, L.F. Lue, D.G. Walker, P. Kuppusamy, Z.L. Zewier, O. Arancio, D. Stern, S.S. Yan, H. Wu, ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease, Science 304 (2004) 448–452.

[15] M. Sastre, T. Klockgether, M.T. Heneka, Contribution of inflammatory processes to Alzheimer's disease: molecular mechanisms, Int. J. Dev. Neurosci. 24 (2006) 167–176.

[16] T. Wyss-Coray, J.D. Loike, T.C. Brionne, E. Lu, R. Anankov, F. Yan, S.C. Silverstein, J. Husemann, Adult mouse astrocytes degrade amyloid-beta in vitro and in situ, Nat. Med. 9 (2003) 453–457.

[17] E.E. Tuppo, H.R. Arias, The role of inflammation in Alzheimer's disease, Int. J. Biochem. Cell Biol. 37 (2005) 289–305.

[18] D.T. Weldon, S.D. Rogers, J.R. Ghilardi, M.P. Finke, J.P. Cleary, E. O'Hare, W.P. Esler, J.E. Maggio, P.W. Mantyh, Fibrillar beta-amyloid induces microglial phagocytosis, expression of inducible nitric oxide synthase, and loss of a select population of neurons in the rat CNS in vivo, J. Neurosci. 18 (1998) 2161–2173.

[19] G.J. Guillemin, K.R. Williams, D.G. Smith, G.A. Smythe, J. Croitoru-Lamoury, B.J. Brew, Quinolinic acid in the pathogenesis of Alzheimer's disease, Adv. Exp. Med. Biol. 527 (2003) 167–176.

[20] N. Kitaguchi, Y. Takahashi, Y. Tokushima, S. Shiojiri, H. Ito, Novel precursor of Alzheimer's disease amyloid protein shows protease inhibitory activity, Nature 331 (1988) 530–532.

[21] M. Mullan, F. Crawford, K. Axelman, H. Houlden, L. Lilius, B. Winblad, L. Lannfelt, A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid, Nat. Genet. 1 (1992) 345–347.

[22] B. De Strooper, L. Umans, F. Van Leuven, H. Van Den Berghe, Study of the synthesis and secretion of normal and artificial mutants of murine amyloid precursor protein (APP): cleavage of APP occurs in a late compartment of the default secretion pathway, J. Cell Biol. 121 (1993) 295–304.

[23] M.D. Kirkitadze, A. Kowalska, Molecular mechanisms initiating amyloid beta-fibril formation in Alzheimer's disease, Acta Biochim. Pol. 52 (2005) 417–423.

[24] M.P. Cuajungco, K.Y. Faget, Zinc takes the center stage: its paradoxical role in Alzheimer's disease, Brain Res. Brain Res. Rev. 41 (2003) 44–56.

[25] K.N. Dahlgren, A.M. Manelli, W.B. Stine Jr., L.K. Baker, G.A. Krafft, M. J. LaDu, Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability, J. Biol. Chem. 277 (2002) 32046–32053.

[26] T.D. Bird, Genetic factors in Alzheimer's disease, N. Engl. J. Med. 352 (2005) 862–864.

[27] A. Capell, H. Steiner, H. Romig, S. Keck, M. Baader, M.G. Grim, R. Baumeister, C. Haass, Presenilin-1 differentially facilitates endoproteolysis of the beta-amyloid precursor protein and Notch, Nat. Cell Biol. 2 (2000) 205–211.

[28] E.H. Corder, A.M. Saunders, W.J. Strittmatter, D.E. Schmechel, P.C. Gaskell, G.W. Small, A.D. Roses, J.L. Haines, M.A. Pericak-Vance, Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families, Science 261 (1993) 921–923.

[29] A. Kowalska, Genetic basis of neurodegeneration in familial Alzheimer's disease, Pol. J. Pharmacol. 56 (2004) 171–178.

[30] Cruts, M.a.R., R., Vol. 2006 August 2006.

[31] D.J. Selkoe, Deciphering the genesis and fate of amyloid beta-protein yields novel therapies for Alzheimer disease, J. Clin. Invest. 110 (2002) 1375–1381.

[32] T.E. Golde, The Abeta hypothesis: leading us to rationally-designed therapeutic strategies for the treatment or prevention of Alzheimer disease, Brain Pathol. 15 (2005) 84–87.

[33] J. Theuns, E. Marjaux, M. Vandenbulcke, K. Van Laere, S. Kumar-Singh, G. Bormans, N. Brouwers, M. Van den Broeck, K. Vennekens, E. Corsmit, M. Cruts, B. De Strooper, C. Van Broeckhoven, R. Vandenberghe, Alzheimer dementia caused by a novel mutation located in the APP C-terminal intracytosolic fragment, Hum. Mutat. 27 (2006) 888–896.

[34] E.S. Walker, M. Martinez, A.L. Brunkan, A. Goate, Presenilin 2 familial Alzheimer's disease mutations result in partial loss of function and dramatic changes in Abeta 42/40 ratios, J. Neurochem. 92 (2005) 294–301.

[35] Q. Chen, D. Schubert, Presenilin-interacting proteins, Expert Rev. Mol. Med. 2002 (2002) 1–18.

[36] C. Haass, C.A. Lemere, A. Capell, M. Citron, P. Seubert, D. Schenk, L. Lannfelt, D.J. Selkoe, The Swedish mutation causes early-onset Alzheimer's disease by beta-secretase cleavage within the secretory pathway, Nat. Med. 1 (1995) 1291–1296.

[37] D.M. Walsh, D.M. Hartley, M.M. Condron, D.J. Selkoe, D.B. Teplow, In vitro studies of amyloid beta-protein fibril assembly and toxicity provide clues to the aetiology of Flemish variant (Ala692–(Gly) Alzheimer's disease, Biochem. J. 355 (2001) 869–877.

[38] M.D. Kirkitadze, M.M. Condron, D.B. Teplow, Identification and characterization of key kinetic intermediates in amyloid beta-protein fibrillogenesis, J. Mol. Biol. 312 (2001) 1103–1119.

[39] Z.Z. Chong, F. Li, K. Maiese, Stress in the brain: novel cellular

mechanisms of injury linked to Alzheimer's disease, Brain Res. Brain Res. Rev. 49 (2005) 1–21.

[40] M.A. Smith, K. Hirai, K. Hsiao, M.A. Pappolla, P.L. Harris, S.L. Siedlak, M. Tabaton, G. Perry, Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress, J. Neurochem. 70 (1998) 2212–2215.

[41] M. Stefani, C.M. Dobson, Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution, J. Mol. Med. 81 (2003) 678–699.

[42] M. Sjogren, K. Blennow, The link between cholesterol and Alzheimer's disease, World J. Biol. Psychiatry 6 (2005) 85–97.

[43] R. Cacabelos, L. Fernandez-Novoa, V. Lombardi, L. Corzo, V. Pichel, Y. Kubota, Cerebrovascular risk factors in Alzheimer's disease: brain hemodynamics and pharmacogenomic implications, Neurol. Res. 25 (2003) 567–580.

[44] A. Hofman, A. Ott, M.M. Breteler, M.L. Bots, A.J. Slooter, F. van Harskamp, M.A. van Duijn, C. Van Broeckhoven, D.E. Grobbee, Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study, Lancet. 349 (1997) 151–154.

[45] A. Ott, R.P. Stolk, A. Hofman, F. van Harskamp, D.E. Grobbee, M.M. Breteler, Association of diabetes mellitus and dementia: the Rotterdam Study, Diabetologia 39 (1996) 1392–1397.

[46] K. Balakrishnan, G. Verdile, P.D. Mehta, J. Beilby, D. Nolan, D.A. Galvao, R. Newton, S.E. Gandy, R.N. Martins, Plasma Abeta42 correlates positively with increased body fat in healthy individuals, J. Alzheimers Dis. 8 (2005) 269–282.

[47] B.V. Zlokovic, Neurovascular mechanisms of Alzheimer's neurodegeneration, Trends Neurosci. 28 (2005) 202–208.

[48] A.J. Dwork, E.A. Schon, J. Herbert, Nonidentical distribution of transferrin and ferric iron in human brain, Neuroscience 27 (1988) 333–345.

[49] C.J. Frederickson, Neurobiology of zinc and zinc-containing neurons, Int. Rev. Neurobiol. 31 (1989) 145–238.

[50] T.E. Droste, R.M. Smith, Neurobiology of Trace Elements, vol. 1, Humana Press, Clifton, NJ, 1983.

[51] S.L. Sensi, H.Z. Yin, S.G. Carriedo, S.S. Rao, J.H. Weiss, Preferential Zn2+influx through Ca2+-permeable AMPA/kainate channels triggers prolonged mitochondrial superoxide production, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 2414–2419.

[52] C.R. Cornett, W.R. Markesbery, W.D. Ehmann, Imbalances of trace elements related to oxidative damage in Alzheimer's disease brain, Neurotoxicology 19 (1998) 339–345.

[53] R. Sherrington, E.I. Rogaev, Y. Liang, E.A. Rogaeva, G. Levesque, M. Ikeda, H. Chi, C. Lin, G. Li, K. Holman, et al., Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease, Nature 375 (1995) 754–760.

[54] E. Levy-Lahad, W. Wasco, P. Poorkaj, D.M. Romano, J. Oshima, W.H. Pettingell, C.E. Yu, P.D. Jondro, S.D. Schmidt, K. Wang, et al., Candidate gene for the chromosome 1 familial Alzheimer's disease locus, Science 269 (1995) 973–977.

[55] E.I. Rogaev, R. Sherrington, E.A. Rogaeva, G. Levesque, M. Ikeda, Y. Liang, H. Chi, C. Lin, K. Holman, T. Tsuda, et al., Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene, Nature 376 (1995) 775–778.

[56] J.H. Chyung, D.M. Raper, D.J. Selkoe, gamma-secretase exists on the plasma membrane as an intact complex that accepts substrates and effects intramembrane cleavage, J. Biol. Chem. 280 (2005) 4383–4392.

[57] B. De Strooper, M. Beullens, B. Contreras, L. Levesque, K. Craessaerts, B. Cordell, D. Moechars, M. Bollen, P. Fraser, P.S. George-Hyslop, F. Van Leuven, Phosphorylation, subcellular localization, and membrane orientation of the Alzheimer's disease-associated presenilins, J. Biol. Chem. 272 (1997) 3590–3598.

[58] L. Holcomb, M.N. Gordon, E. McGowan, X. Yu, S. Benkovic, P. Jantzen, K. Wright, I. Saad, R. Mueller, D. Morgan, S. Sanders, C. Zehr, K. O'Campo, J. Hardy, C.M. Prada, C. Eckman, S. Younkin, K. Hsiao, K. Duff, Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes, Nat. Med. 4 (1998) 97–100.

[59] M. Citron, D. Westaway, W. Xia, G. Carlson, T. Diehl, G. Levesque, K. Johnson-Wood, M. Lee, P. Seubert, A. Davis, D. Kholodenko, R. Motter, R. Sherrington, B. Perry, H. Yao, R. Strome, I. Lieberburg, J. Rommens, S. Kim, D. Schenk, P. Fraser, P. St George Hyslop, D.J. Selkoe, Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice, Nat. Med. 3 (1997) 67–72.

[60] D. Scheuner, C. Eckman, M. Jensen, X. Song, M. Citron, N. Suzuki, T.D. Bird, J. Hardy, M. Hutton, W. Kukull, E. Larson, E. Levy-Lahad, M. Viitanen, E. Peskind, P. Poorkaj, G. Schellenberg, R. Tanzi, W. Wasco, L. Lannfelt, D. Selkoe, S. Younkin, Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease, Nat. Med. 2 (1996) 864–870.

[61] B. De Strooper, P. Saftig, K. Craessaerts, H. Vanderstichele, G. Guhde, W. Annaert, K. Von Figura, F. Van Leuven, Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein, Nature 391 (1998) 387–390.

[62] A. Herreman, D. Hartmann, W. Annaert, P. Saftig, K. Craessaerts, L. Serneels, L. Umans, V. Schrijvers, F. Checler, H. Vanderstichele, V. Baekelandt, R. Dressel, P. Cupers, D. Huylebroeck, A. Zwijsen, F. Van Leuven, B. De Strooper, Presenilin 2 deficiency causes a mild pulmonary phenotype and no changes in amyloid precursor protein processing but enhances the embryonic lethal phenotype of presenilin 1 deficiency, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 11872–11877.

[63] R. Feng, H. Wang, J. Wang, D. Shrom, X. Zeng, J.Z. Tsien, Forebrain degeneration and ventricle enlargement caused by double knockout of Alzheimer's presenilin-1 and presenilin-2, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 8162–8167.

[64] M.J. Smith, R.A. Sharples, G. Evin, C.A. McLean, B. Dean, G. Pavey, E. Fantino, R.G. Cotton, K. Imaizumi, C.L. Masters, R. Cappai, J.G. Culvenor, Expression of truncated presenilin 2 splice variant in Alzheimer's disease, bipolar disorder, and schizophrenia brain cortex, Brain Res. Mol. Brain Res. 127 (2004) 128–135.

[65] A.L. Brunkan, A.M. Goate, Presenilin function and gamma-secretase activity, J. Neurochem. 93 (2005) 769–792.

[66] K.S. Vetrivel, Y.W. Zhang, H. Xu, G. Thinakaran, Pathological and physiological functions of presenilins, Mol. Neurodegener. 1 (2006) 4.

[67] H. Tu, O. Nelson, A. Bezprozvanny, Z. Wang, S.F. Lee, Y.H. Hao, L. Serneels, B. De Strooper, G. Yu, I. Bezprozvanny, Presenilins form ER Ca2+leak channels, a function disrupted by familial Alzheimer's disease-linked mutations, Cell 126 (2006) 981–993.

[68] P. Vito, E. Lacana, L. D'Adamio, Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3, Science 271 (1996) 521–525.

[69] A. Alberici, D. Moratto, L. Benussi, L. Gasparini, R. Ghidoni, L.B. Gatta, D. Finazzi, G.B. Frisoni, M. Trabucchi, J.H. Growdon, R.M. Nitsch, G. Binetti, Presenilin 1 protein directly interacts with Bcl-2, J. Biol. Chem. 274 (1999) 30764–30769.

[70] B.J. Passer, L. Pellegrini, P. Vito, J.K. Ganjei, L. D'Adamio, Interaction of Alzheimer's presenilin-1 and presenilin-2 with Bcl-X(L). A potential role in modulating the threshold of cell death, J. Biol. Chem. 274 (1999) 24007–24013.

[71] X. Li, I. Greenwald, Additional evidence for an eight-transmembrane-domain topology for *Caenorhabditis elegans* and human presenilins, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 7109–7114.

[72] N.N. Dewji, S.J. Singer, The seven-transmembrane spanning topography of the Alzheimer disease-related presenilin proteins in the plasma membranes of cultured cells, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 14025–14030.

[73] S. Lehmann, R. Chiesa, D.A. Harris, Evidence for a six-transmembrane domain structure of presenilin 1, J. Biol. Chem. 272 (1997) 12047–12051.

[74] D. Spasic, A. Tolia, K. Dillen, V. Baert, B. De Strooper, S. Vrijens, W.

Annaert, Presenilin-1 maintains a nine transmembrane topology throughout the secretory pathway, J. Biol. Chem. (2006).

[75] H. Laudon, E.M. Hansson, K. Melen, A. Bergman, M.R. Farmery, B. Winblad, U. Lendahl, G. von Heijne, J. Naslund, A nine-transmembrane domain topology for presenilin 1, J. Biol. Chem. 280 (2005) 35352–35360.

[76] M.S. Wolfe, W. Xia, B.L. Ostaszewski, T.S. Diehl, W.T. Kimberly, D.J. Selkoe, Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity, Nature 398 (1999) 513–517.

[77] W.T. Kimberly, W. Xia, T. Rahmati, M.S. Wolfe, D.J. Selkoe, The transmembrane aspartates in presenilin 1 and 2 are obligatory for gamma-secretase activity and amyloid beta-protein generation, J. Biol. Chem. 275 (2000) 3173–3178.

[78] M.B. Podlisny, M. Citron, P. Amarante, R. Sherrington, W. Xia, J. Zhang, T. Diehl, G. Levesque, P. Fraser, C. Haass, E.H. Koo, P. Seubert, P. St George-Hyslop, D.B. Teplow, D.J. Selkoe, Presenilin proteins undergo heterogeneous endoproteolysis between Thr291 and Ala299 and occur as stable N- and C-terminal fragments in normal and Alzheimer brain tissue, Neurobiol. Dis. 3 (1997) 325–337.

[79] G. Thinakaran, D.R. Borchelt, M.K. Lee, H.H. Slunt, L. Spitzer, G. Kim, T. Ratovitsky, F. Davenport, C. Nordstedt, M. Seeger, J. Hardy, A.I. Levey, S.E. Gandy, N.A. Jenkins, N.G. Copeland, D.L. Price, S.S. Sisodia, Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo, Neuron 17 (1996) 181–190.

[80] T. Ratovitski, H.H. Slunt, G. Thinakaran, D.L. Price, S.S. Sisodia, D.R. Borchelt, Endoproteolytic processing and stabilization of wild-type and mutant presenilin, J. Biol. Chem. 272 (1997) 24536–24541.

[81] H. Steiner, A. Capell, B. Pesold, M. Citron, P.M. Kloetzel, D.J. Selkoe, H. Romig, K. Mendla, C. Haass, Expression of Alzheimer's disease-associated presenilin-1 is controlled by proteolytic degradation and complex formation, J. Biol. Chem. 273 (1998) 32322–32331.

[82] A. Capell, J. Grunberg, B. Pesold, A. Diehlmann, M. Citron, R. Nixon, K. Beyreuther, D.J. Selkoe, C. Haass, The proteolytic fragments of the Alzheimer's disease-associated presenilin-1 form heterodimers and occur as a 100–150-kDa molecular mass complex, J. Biol. Chem. 273 (1998) 3205–3211.

[83] S.S. Hebert, C. Godin, G. Levesque, Oligomerization of human presenilin-1 fragments, FEBS Lett. 550 (2003) 30–34.

[84] C. Haass, H. Steiner, Alzheimer disease gamma-secretase: a complex story of GxGD-type presenilin proteases, Trends Cell Biol. 12 (2002) 556–562.

[85] S. Cervantes, R. Gonzalez-Duarte, G. Marfany, Homodimerization of presenilin N-terminal fragments is affected by mutations linked to Alzheimer's disease, FEBS Lett. 505 (2001) 81–86.

[86] M.S. Brown, J. Ye, R.B. Rawson, J.L. Goldstein, Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans, Cell 100 (2000) 391–398.

[87] W.T. Kimberly, M.J. LaVoie, B.L. Ostaszewski, W. Ye, M.S. Wolfe, D.J. Selkoe, Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 6382–6387.

[88] W.T. Kimberly, W.P. Esler, W. Ye, B.L. Ostaszewski, J. Gao, T. Diehl, D.J. Selkoe, M.S. Wolfe, Notch and the amyloid precursor protein are cleaved by similar gamma-secretase(s), Biochemistry 42 (2003) 137–144.

[89] N. Takasugi, T. Tomita, I. Hayashi, M. Tsuruoka, M. Niimura, Y. Takahashi, G. Thinakaran, T. Iwatsubo, The role of presenilin cofactors in the gamma-secretase complex, Nature 422 (2003) 438–441.

[90] Y. Hu, Y. Ye, M.E. Fortini, Nicastrin is required for gamma-secretase cleavage of the *Drosophila* Notch receptor, Dev. Cell 2 (2002) 69–78.

[91] G. Yu, M. Nishimura, S. Arawaka, D. Levitan, L. Zhang, A. Tandon, Y.Q. Song, E. Rogaeva, F. Chen, T. Kawarai, A. Supala, L. Levesque, H. Yu, D.S. Yang, E. Holmes, P. Milman, Y. Liang, D.M. Zhang, D.H. Xu, C. Sato, E. Rogaev, M. Smith, C. Janus, Y. Zhang, R. Aebersold, L.S. Farrer, S. Sorbi, A. Bruni, P. Fraser, P. St George-Hyslop, Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing, Nature 407 (2000) 48–54.

[92] T. Li, G. Ma, H. Cai, D.L. Price, P.C. Wong, Nicastrin is required for assembly of presenilin/gamma-secretase complexes to mediate Notch signaling and for processing and trafficking of beta-amyloid precursor protein in mammals, J. Neurosci. 23 (2003) 3272–3277.

[93] Y.W. Zhang, W.J. Luo, H. Wang, P. Lin, K.S. Vetrivel, F. Liao, F. Li, P.C. Wong, M.G. Farquhar, G. Thinakaran, H. Xu, Nicastrin is critical for stability and trafficking but not association of other presenilin/gamma-secretase components, J. Biol. Chem. 280 (2005) 17020–17026.

[94] F. Chen, H. Hasegawa, G. Schmitt-Ulms, T. Kawarai, C. Bohm, T. Katayama, Y. Gu, N. Sanjo, M. Glista, E. Rogaeva, Y. Wakutani, R. Pardossi-Piquard, X. Ruan, A. Tandon, F. Checler, P. Marambaud, K. Hansen, D. Westaway, P. St George-Hyslop, P. Fraser, TMP21 is a presenilin complex component that modulates gamma-secretase but not epsilon-secretase activity, Nature 440 (2006) 1208–1212.

[95] H. Steiner, M. Kostka, H. Romig, G. Basset, B. Pesold, J. Hardy, A. Capell, L. Meyn, M.L. Grim, R. Baumeister, K. Fechteler, C. Haass, Glycine 384 is required for presenilin-1 function and is conserved in bacterial polytopic aspartyl proteases, Nat. Cell Biol. 2 (2000) 848–851.

[96] E.H. Schroeter, J.A. Kisslinger, R. Kopan, Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain, Nature 393 (1998) 382–386.

[97] G. Struhl, I. Greenwald, Presenilin is required for activity and nuclear access of Notch in *Drosophila*, Nature 398 (1999) 522–525.

[98] D. Levitan, T.G. Doyle, D. Brousseau, M.K. Lee, G. Thinakaran, H.H. Slunt, S.S. Sisodia, I. Greenwald, Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 14940–14944.

[99] E.H. Koo, R. Kopan, Potential role of presenilin-regulated signaling pathways in sporadic neurodegeneration, Nat. Med. 10 Suppl (2004) S26–S33.

[100] P.E. Fraser, D.S. Yang, G. Yu, L. Levesque, M. Nishimura, S. Arawaka, L.C. Serpell, E. Rogaeva, P. St George-Hyslop, Presenilin structure, function and role in Alzheimer disease, Biochim. Biophys. Acta 1502 (2000) 1–15.

[101] N. Sato, O. Hori, A. Yamaguchi, J.C. Lambert, M.C. Chartier-Harlin, P.A. Robinson, A. Delacourte, A.M. Schmidt, T. Furuyama, K. Imaizumi, M. Tohyama, T. Takagi, A novel presenilin-2 splice variant in human Alzheimer's disease brain tissue, J. Neurochem. 72 (1999) 2498–2505.

[102] S. Higashide, K. Morikawa, M. Okumura, S. Kondo, M. Ogata, T. Murakami, A. Yamashita, S. Kanemoto, T. Manabe, K. Imaizumi, Identification of regulatory cis-acting elements for alternative splicing of presenilin 2 exon 5 under hypoxic stress conditions, J. Neurochem. 91 (2004) 1191–1198.

[103] S. Matsuzaki, T. Manabe, T. Katayama, A. Nishikawa, T. Yanagita, H. Okuda, Y. Yasuda, S. Miyata, S. Meshitsuka, M. Tohyama, Metals accelerate production of the aberrant splicing isoform of the presenilin-2, J. Neurochem. 88 (2004) 1345–1351.

[104] J.B. Kwok, G.M. Halliday, W.S. Brooks, G. Dolios, H. Laudon, O. Murayama, M. Hallupp, R.F. Badenhop, J. Vickers, R. Wang, J. Naslund, A. Takashima, S.E. Gandy, P.R. Schofield, Presenilin-1 mutation L271V results in altered exon 8 splicing and Alzheimer's disease with non-cored plaques and no neuritic dystrophy, J. Biol. Chem. 278 (2003) 6748–6754.

[105] R. Crook, A. Verkkoniemi, J. Perez-Tur, N. Mehta, M. Baker, H. Houlden, M. Farrer, M. Hutton, S. Lincoln, J. Hardy, K. Gwinn, M. Somer, A. Paetau, H. Kalimo, R. Ylikoski, M. Poyhonen, S. Kucera, M. Haltia, A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1, Nat. Med. 4 (1998) 452–455.

[106] G. Evin, M.J. Smith, A. Tziotis, C. McLean, L. Canterford, R.A. Sharples, R. Cappai, A. Weidemann, K. Beyreuther, R.G. Cotton, C.L. Masters, J.G. Culvenor, Alternative transcripts of presenilin-1 associated with frontotemporal dementia, NeuroReport 13 (2002) 917–921.

[107] B. Dermaut, S. Kumar-Singh, S. Engelborghs, J. Theuns, R. Rademakers, J. Saerens, B.A. Pickut, K. Peeters, M. van den Broeck, K. Vennekens, S. Claes, M. Cruts, P. Cras, J.J. Martin, C. Van Broeckhoven, P.P. De Deyn, A novel presenilin 1 mutation associated with Pick's disease but not beta-amyloid plaques, Ann. Neurol. 55 (2004) 617–626.

[108] F. Assal, R. Sztajzel, A. Carota, J.M. Annoni, J. Bogousslavsky.

Neurodegeneration and cerebrovascular disease: causal or incidental link? Rev. Med. Suisse 2 (2006) 1180–82, 1184.

[109] A. Rovelet-Lecrux, D. Hannequin, G. Raux, N. Le Meur, A. Laquerriere, A. Vital, C. Dumanchin, S. Feuillette, A. Brice, M. Vercelletto, F. Dubas, T. Frebourg, D. Campion, APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy, Nat. Genet. 38 (2006) 24–26.

[110] A. Joutel, K. Vahedi, C. Corpechot, A. Troesch, H. Chabriat, C. Vayssiere, C. Cruaud, J. Maciazek, J. Weissenbach, M.G. Bousser, J.F. Bach, E. Tournier-Lasserve, Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients, Lancet. 350 (1997) 1511–1515.

[111] M. Vikelis, M. Xifaras, D.D. Mitsikostas, CADASIL: a short review of the literature and a description of the first family from Greece, Funct. Neurol. 21 (2006) 77–82.

[112] J.E. Meredith Jr., Q. Wang, T.J. Mitchell, R.E. Olson, R. Zaczek, A.M. Stern, D. Seiffert, Gamma-secretase activity is not involved in presenilin-mediated regulation of beta-catenin, Biochem. Biophys. Res. Commun. 299 (2002) 744–750.

[113] B. De Strooper, W. Annaert, Where Notch and Wnt signaling meet. The presenilin hub, J. Cell Biol. 152 (2001) F17–F20.

[114] E. Noll, M. Medina, D. Hartley, J. Zhou, N. Perrimon, K.S. Kosik, Presenilin affects arm/beta-catenin localization and function in *Drosophila*, Dev. Biol. 227 (2000) 450–464.

[115] S. Soriano, D.E. Kang, M. Fu, R. Pestell, N. Chevallier, H. Zheng, E. H. Koo, Presenilin 1 negatively regulates beta-catenin/T cell factor/lymphoid enhancer factor-1 signaling independently of beta-amyloid precursor protein and notch processing, J. Cell Biol. 152 (2001) 785–794.

[116] M. Murayama, S. Tanaka, J. Palacino, O. Murayama, T. Honda, X. Sun, K. Yasutake, N. Nihonmatsu, B. Wolozin, A. Takashima, Direct association of presenilin-1 with beta-catenin, FEBS Lett. 433 (1998) 73–77.

[117] Z. Zhang, H. Hartmann, V.M. Do, D. Abramowski, C. Sturchler-Pierrat, M. Staufenbiel, B. Sommer, M. van de Wetering, H. Clevers, P. Saftig, B. De Strooper, X. He, B.A. Yankner, Destabilization of beta-catenin by mutations in presenilin-1 potentiates neuronal apoptosis, Nature 395 (1998) 698–702.

[118] D.E. Kang, S. Soriano, M.P. Frosch, T. Collins, S. Naruse, S.S. Sisodia, G. Leibowitz, F. Levine, E.H. Koo, Presenilin 1 facilitates the constitutive turnover of beta-catenin: differential activity of Alzheimer's disease-linked PS1 mutants in the beta-catenin-signaling pathway, J. Neurosci. 19 (1999) 4229–4237.

[119] U. Wagner, J. Brownlees, N.G. Irving, F.R. Lucas, P.C. Salinas, C.C. Miller, Overexpression of the mouse dishevelled-1 protein inhibits GSK-3beta-mediated phosphorylation of tau in transfected mammalian cells, FEBS Lett. 411 (1997) 369–372.

[120] U. Leimer, K. Lun, H. Romig, J. Walter, J. Grunberg, M. Brand, C. Haass, Zebrafish (*Danio rerio*) presenilin promotes aberrant amyloid beta-peptide production and requires a critical aspartate residue for its function in amyloidogenesis, Biochemistry 38 (1999) 13602–13609.

[121] C. Groth, S. Nornes, R. McCarty, R. Tamme, M. Lardelli, Identification of a second presenilin gene in zebrafish with similarity to the human Alzheimer's disease gene presenilin2, Dev. Genes Evol. 212 (2002) 486–490.

[122] S. Nornes, C. Groth, E. Camp, P. Ey, M. Lardelli, Developmental control of Presenilin1 expression, endoproteolysis, and interaction in zebrafish embryos, Exp. Cell Res. 289 (2003) 124–132.

[123] D.C. Crowther, K.J. Kinghorn, E. Miranda, R. Page, J.A. Curry, F.A. Duthie, D.C. Gubb, D.A. Lomas, Intraneuronal Abeta, non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease, Neuroscience 132 (2005) 123–135.

[124] A. Finelli, A. Kelkar, H.J. Song, H. Yang, M. Konsolaki, A model for studying Alzheimer's Abeta42-induced toxicity in *Drosophila melanogaster*, Mol. Cell Neurosci. 26 (2004) 365–375.

[125] X. Li, I. Greenwald, HOP-1, a *Caenorhabditis elegans* presenilin, appears to be functionally redundant with SEL-12 presenilin and to facilitate LIN-12 and GLP-1 signaling, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 12204–12209.

[126] P.M. Arduengo, O.K. Appleberry, P. Chuang, S.W. L'Hernault, The presenilin protein family member SPE-4 localizes to an ER/Golgi derived organelle and is required for proper cytoplasmic partitioning during *Caenorhabditis elegans* spermatogenesis, J. Cell Sci. 111 (Pt 24) (1998) 3645–3654.

[127] S.W. L'Hernault, P.M. Arduengo, Mutation of a putative sperm membrane protein in *Caenorhabditis elegans* prevents sperm differentiation but not its associated meiotic divisions, J. Cell Biol. 119 (1992) 55–68.

[128] C.D. Link, Expression of human beta-amyloid peptide in transgenic *Caenorhabditis elegans*, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 9368–9372.

[129] S. Oddo, A. Caccamo, M. Kitazawa, B.P. Tseng, F.M. LaFerla, Amyloid deposition precedes tangle formation in a triple transgenic model of Alzheimer's disease, Neurobiol. Aging 24 (2003) 1063–1070.

[130] L.M. Billings, S. Oddo, K.N. Green, J.L. McGaugh, F.M. Laferla, Intraneuronal Abeta causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice, Neuron 45 (2005) 675–688.

[131] K.H. Ashe, Mechanisms of memory loss in Abeta and tau mouse models, Biochem. Soc. Trans. 33 (2005) 591–594.

[132] Y. Tatebayashi, T. Miyasaka, D.H. Chui, T. Akagi, K. Mishima, K. Iwasaki, M. Fujiwara, K. Tanemura, M. Murayama, K. Ishiguro, E. Planel, S. Sato, T. Hashikawa, A. Takashima, Tau filament formation and associative memory deficit in aged mice expressing mutant (R406W) human tau, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 13896–13901.

[133] H.G. Tomasiewicz, D.B. Flaherty, J.P. Soria, J.G. Wood, Transgenic zebrafish model of neurodegeneration, J. Neurosci. Res. 70 (2002) 734–745.

[134] N. Scheer, J.A. Campos-Ortega, Use of the Gal4-UAS technique for targeted gene expression in the zebrafish, Mech. Dev. 80 (1999) 153–158.

[135] H. Steiner, E. Winkler, D. Edbauer, S. Prokop, G. Basset, A. Yamasaki, M. Kostka, C. Haass, PEN-2 is an integral component of the gamma-secretase complex required for coordinated expression of presenilin and nicastrin, J. Biol. Chem. 277 (2002) 39062–39065.

[136] W.A. Campbell, H. Yang, H. Zetterberg, S. Baulac, J.A. Sears, T. Liu, S. T. Wong, T.P. Zhong, W. Xia, Zebrafish lacking Alzheimer presenilin enhancer 2 (Pen-2) demonstrate excessive p53-dependent apoptosis and neuronal loss, J. Neurochem. 96 (2006) 1423–1440.

[137] H.F. Dovey, V. John, J.P. Anderson, L.Z. Chen, P. de Saint, L.Y. de Saint Andrieu, S.B. Fang, B. Freedman, E. Folmer, E.J. Goldbach, K.L. Holsztynska, K.L. Hu, S.L. Johnson-Wood, D. Kennedy, J.E. Kholodenko, L.H. Knops, M. Latimer, Z. Lee, I.M. Liao, R.N. Lieberburg, L.C. Motter, J. Mutter, K.P. Nietz, K.L. Quinn, P.A. Sacchi, G.M. Seubert, E.D. Shopp, J.S. Thorsett, J. Tung, S. Wu, C.T. Yang, D.B. Yin, P.C. Schenk, L.D. May, M.H. Altstiel, L.N. Bender, T.C. Boggs, J.C. Britton, D.L. Clemens, D.K. Czilli, J.J. Dieckman-McGinty, K.S. Droste, B.D. Fuson, P.A. Gitter, E.M. Hyslop, W.Y. Johnstone, S.P. Li, T.E. Little, F.D. Mabry, J.E. Miller, Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain, J. Neurochem. 76 (2001) 173–181.

[138] A. Geling, H. Steiner, M. Willem, L. Bally-Cuif, C. Haass, A gamma-secretase inhibitor blocks Notch signaling in vivo and causes a severe neurogenic phenotype in zebrafish, EMBO Rep. 3 (2002) 688–694.

[139] D.B. Donoviel, A.K. Hadjantonakis, M. Ikeda, H. Zheng, P.S. Hyslop, A. Bernstein, Mice lacking both presenilin genes exhibit early embryonic patterning defects, Genes Dev. 13 (1999) 2801–2810.

[140] G. Thinakaran, C.L. Harris, T. Ratovitski, F. Davenport, H.H. Slunt, D.L. Price, D.R. Borchelt, S.S. Sisodia, Evidence that levels of presenilins (PS1 and PS2) are coordinately regulated by competition for limiting cellular factors, J. Biol. Chem. 272 (1997) 28415–28422.

[141] Nornes, S.a.L., M. Manuscript in progress.

[142] A. Musa, H. Lehrach, V.A. Russo, Distinct expression patterns of two zebrafish homologues of the human APP gene during embryonic development, Dev. Genes Evol. 211 (2001) 563–567.

[143] M. Sarasa, V. Sorribas, J. Terradoa, S. Climent, J.M. Palacios, G. Mengod, Alzheimer beta-amyloid precursor proteins display specific patterns of expression during embryogenesis, Mech. Dev. 94 (2000) 233–236.

[144] M.J. Carvan III, D.M. Sonntag, C.B. Cmar, R.S. Cook, M.A. Curran, G.L. Miller, Oxidative stress in zebrafish cells: potential utility of transgenic zebrafish as a deployable sentinel for site hazard ranking, Sci. Total Environ. 274 (2001) 183–196.